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VITAMIN A CONTENT OF CUBAN COW'S MILK AND OF LIVER OILS OF CUBAN SEA SHARKS

JUAN J. ANGULO, RAÚL F. COWLEY, ALBERTO MARRERO, AND CÉSAR FUENTES

Department of Experimental Pathology, School of Medicine, Havana University and Laboratory of Compañía de Pesca del Valle, S. A., Havana, Cuba

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Continuing the work of determining the vitamin A content of Cuban foods started by Angulo, Fuentes, and Johnson (1946), determinations were made of the vitamin A and carotene content of cow's milk and of the vitamin A content of liver oils obtained from sharks found in the waters of Cuba. The results are reported in this paper.

Besides the known fact that chemical compositions of foods vary from one country to another, the above-mentioned work was necessary because most of the milk consumed in Cuba is produced by cows of a very mixed stock adapted not only to the Cuban climate but to a deficient diet, and food composition influences the vitamin A content of milk, as shown by Fraps, Copeland, and Treichler (1934). Besides, since most of the milk consumed by the Cuban people is previously boiled to prevent infections, the vitamin A content of boiled milk as compared with raw and pasteurized milk was considered important information.

EXPERIMENTAL PROCEDURE

Fifteen samples of cow's milk were taken from different Havana cafeterias so that the actual values of vitamin A and carotene could be determined. The samples were taken during the late summer and the fall.

Six samples were of Grade A milk and nine of Grade B milk; three of the six Grade A milk samples were of boiled milk, the remaining three of raw milk, while the nine Grade B milk samples were composed of three samples of each type of milk boiled, pasteurized, and raw. Grade A milk is not regularly pasteurized in Cuba.

The vitamin A and carotene determinations in milk samples (Table 1) were performed according to the method of Boyer, Spitzer, Jensen, and Phillips (1944). A Model B Pfaltz and Bauer fluorometer was calibrated with Wyeth beta-carotene and with the distilled vitamin A concentrate of Distillation Products Company known as the PC-3 concentrate.

Forty-six samples of shark-liver oils and one sample of spotted whip ray-liver oil obtained from a factory for extracting liver oils from sharks caught in the waters of Cuba were spectrophotometrically assayed in a Model 410 Lumetron photoelectric colorimeter, using iso-propyl alcohol as solvent. The colorimeter was calibrated with a USP Reference cod-liver oil and a shark-liver oil standardized by the Food Research Laboratories, Inc., Long Island City, New York. The figures are shown (Tables 2 and 3).

RESULTS AND DISCUSSION

In the different types of milk assayed the greatest vitamin A and carotene contents were found in the Grade A boiled-milk samples, and the lowest were found in the Grade B raw-milk samples (Table 1). The vitamin A and carotene content values obtained in the three different samples of each type of milk were fairly uniform. The values of each type of milk were graded in the following decreasing order: boiled Grade A milk, raw Grade A milk, boiled Grade B milk, and, finally, raw Grade B milk and pasteurized Grade B milk, as no significant difference was observed between the last two types.

TABLE 1

Vitamin A Content of Cuban Cow's Milk

GRADE A MILK

Raw milk			Boiled milk		
Sample No.	Carotene	Vitamin A	Sample No.	Carotene	Vitamin A
	μg./100 ml.	μg./100 ml.		μg./100 ml.	μg./100 ml.
1	137	135	4	323	206
2	168	123	5	351	111
3	176	138	6	390	212

GRADE B MILK

Raw milk			Pasteurized milk			Boiled milk		
Sample No.	Carotene	Vitamin A	Sample No.	Carotene	Vitamin A	Sample No.	Carotene	Vitamin A
	μg./100 ml.	μg./100 ml.		μg./100 ml.	μg./100 ml.		μg./100 ml.	μg./100 ml.
7	57	53	10	60	45	13	65	78
8	71	30	11	82	42	14	119	53
9	95	53	12	110	50	15	146	88

All the boiled-milk samples showed higher vitamin A and carotene content than the raw-milk or pasteurized-milk samples, and all Grade A milk samples showed higher vitamin A and carotene content than Grade B milk, the difference being important.

The higher values of vitamin A and carotene content in the boiled-milk samples are probably due to the evaporation of water during the boiling process. Hence, the common practice in Cuba of boiling milk to prevent infections has this added advantage of concentrating the vitamin A and carotene per volume of milk. Pasteurization does not represent this advantage.

Boyer, Spitzer, Jensen, and Phillips (1944) determined the vitamin A and carotene content of milk obtained from Guernsey, Holstein, Brown Swiss, and Jersey cows. A comparison of the values found by Boyer *et al.* with the values of the vitamin A and carotene found in the milk of the Cuban mixed-breed cows shows that the Grade A raw milk of the Cuban cows is higher in vitamin A and carotene than that of the Guernsey cow and much higher than that of the Holstein, Brown Swiss, and Jersey cows,

TABLE 2

Vitamin A in Cuban Shark Liver Oils (Industrial Samples)

Common name	Sex	Weight	Scientific name	Vitamin A in oil
		lb.		*I.U./ gm.*
Albacorera	.....	.....	? ?	2,800
Albacorera	.....	.....	? ?	4,800
Tiger shark	Female	.....	*Galeocerdo arcticus* (Faber)	3,000
Tiger shark	Female	.....	*Galeocerdo arcticus* (Faber)	7,500
Smooth dogfish	.....	.....	*Mustelus canis* (Mitchill)	5,300
Smooth dogfish	.....	.....	*Mustelus canis* (Mitchill)	5,700
Cabezón	Female	80	? ?	7,700
Cow shark	.....	.....	*Hexanchus griseus* (Bonnaterre)	450
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	3,300
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	4,600
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	5,300
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	6,100
Hammerhead shark	Female	300	*Sphyrna zygaena* (Linnaeus)	6,300
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	7,000
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	16,250
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	20,800
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	21,250
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	22,000
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	23,000
Hammerhead shark	Male	400	*Sphyrna zygaena* (Linnaeus)	28,500
Hammerhead shark	Male	.....	*Sphyrna zygaena* (Linnaeus)	29,500
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	30,500
Hammerhead shark	Male	536	*Sphyrna zygaena* (Linnaeus)	31,000
Hammerhead shark	Male	280	*Sphyrna zygaena* (Linnaeus)	34,600
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	38,500
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	41,500
Hammerhead shark	.....	.....	*Sphyrna zygaena* (Linnaeus)	60,600
Elefante	.....	.....	? ?	850
Man-eater shark	Female	7,000	*Carcharodon carcharias* (Linnaeus)	1,150
Sharp-nosed mackerel shark	.....	.....	*Isurus tigris* (Atwood)	1,275
Sharp-nosed mackerel shark	.....	.....	*Isurus tigris* (Atwood)	2,100
Spot-fin ground shark	Male	240	*Carcharias limbatus* (Müller & Henle)	11,700
Nurse shark	.....	.....	*Ginglymostoma cirratum* (Gmelin)	2,000
Nurse shark	.....	.....	*Ginglymostoma cirratum* (Gmelin)	3,700
?	.....	.....	*Hexanchus* sp.	1,075
?	Male	826	*Hexanchus* sp.	2,700
?	Female	.....	*Hexanchus* sp.	4,000
?	Male	.....	*Hexanchus* sp.	4,200
Jaquetón	.....	.....	*Carcharias* sp.	3,250
Jaquetón	Female	240	*Carcharias* sp.	5,700
Jaquetón	.....	.....	*Carcharias* sp.	10,350
Jaquetón	.....	.....	*Carcharias* sp.	11,250
Jaquetón	Female	272	*Carcharias* sp.	13,400
Quimbombó	.....	.....	? ?	5,400
Quimbombó	.....	.....	? ?	7,900
Quimbombó	.....	.....	? ?	17,400
Spotted whip ray (a Batoidei, not a Selachii)	.....	.....	*Aëtobatus narinari* (Euphrasen)	145

It is notable that among the sharks studied the liver oils of those commonly known as hammerhead, *quimbombó, jaquetón,* and the female tiger shark are, in decreasing order, the best sources of vitamin A (Table 3). Although the liver oils of spot-fin ground shark and *cabezón* shark showed fair values, only one sample was tested in each case.

The greatest sample content variation in a given species is found in the hammerhead shark, in which the values ranged from 3,300 to 60,600 international units per gram of oil.

With the data at hand, it is impossible to detect a relation between the vitamin A content of the liver oil and sex and weight of specimens of the same species.

TABLE 3

Range of Vitamin A Content of Liver Oils of Shark Species Studied

Species	Vitamin A			
	Minimum		Maximum	Number of samples assayed
	I.U./gm.	*I.U./gm.*	*I.U./gm.*	
Hammerhead shark	3,300	.....	60,600	19
Quimbombó	5,400	.....	17,400	3
Jaquetón	3,250	.....	13,400	5
Tiger shark, female	3,000	.....	7,500	2
Spot-fin ground shark	.....	11,700	.....	1
Cabezón	.....	7,700	.....	1
Smooth dogfish	5,300	.....	5,700	2
Albacorera	2,800	.....	4,800	2
Hexanchus sp	450	.....	4,200	5
Nurse shark	2,000	.....	3,700	2
Sharp-nosed mackerel shark	1,275	.....	2,100	2
Man-eater shark	.....	1,150	.....	1
Elefante	.....	850	.....	1
Spotted whip ray (a Batoidei, not a Selachii)	.....	145	.....	1

In general, the figures found in the present assays are comparable to those of Coy, Sassaman, and Black (1943), but the lowest figure of the range found by them is higher than those found in 16 of the 46 samples assayed by the authors. These 16 samples, as well as the spotted whip ray-liver-oil sample, showed figures characteristic of cod-liver-oil samples. On the other hand, none of the 46 samples of shark-liver oil assayed for vitamin A showed values higher than 100,000 international units per gram, which has been reported by Coy, Sassaman, and Black (1941). The industrial process may be responsible for that.

SUMMARY

Fifteen samples of cow's milk collected from Havana cafeterias during the late summer and the fall were assayed for vitamin A and carotene content. All boiled-milk samples showed higher vitamin A and carotene content than the raw-milk or pasteurized-milk samples, and all Grade A milk samples showed higher vitamin A and carotene content than Grade B milk samples, the difference being great. No significant difference was

found between the values obtained for raw-milk or pasteurized-milk samples of the same grade.

Forty-six shark-liver-oil samples and one sample of spotted whip ray-liver oil, obtained from fish caught in the waters of Cuba were assayed for vitamin A content. Seventeen of the 47 samples gave low values that are comparable to those found in cod-liver oils. The figures obtained in the remaining samples fluctuated through the same range found in the United States for shark-liver oil, although none of the Cuban samples was close to the higher figures of that range. Oils obtained from sharks commonly known as hammerhead, *quimbombó*, *jaquetón*, and female tiger shark showed the highest values among the sharks studied; while those from the smooth dogfish, *albacorera*, *Hexanchus* sp., nurse shark, sharp-nosed mackerel shark, man-eater, and *elefante* gave figures comparable to those of cod-liver oil. The same may be said about the spotted whip ray-liver-oil sample which showed the lowest value found.

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TEEN-AGE STUDENTS VERSUS ADULTS AS TASTE JUDGES OF TEMPLE ORANGES

PAUL L. HARDING

Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering

AND

F. M. WADLEY

Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture

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Harding and Wadley published in 1945 a discussion of grades given Temple oranges by tasters, with statistical analysis and comparison with laboratory determinations (*Food Research* 10, 510-517). Methods used, trends, and limitations of the material were discussed. There was no plot replication for study of fruits nor sampling replication for laboratory determinations. Variations between fruit from different rootstocks, between tasters, and between degrees of maturity were studied. Rootstocks and tasters showed moderate variation, while degree of maturity brought great variation in palatability. Ratings showed a good correlation with laboratory determinations of dissolved solids and acidity.

During 1946 interest in the subject led to further tests. The suggestion that teen-age, high school judges might be preferable to adults was given a trial. Four lots of oranges were tested from two rootstocks (rough lemon and sour orange) in each of two localities (Merritt Island and Lake Wales, Florida). From each lot three samples were drawn and used both in tasting and in laboratory tests. Thus both plot and sample replication were provided. The tests were made all in one day, in February, near the peak of palatability for this variety.

Two groups of judges were employed: 39 adults and 23 high school boys and girls.[1] The adult judges were largely the ones participating in the previous work. Their ages ranged between 19 and 70 with 38 years as the average age. The high school students ranged in age between 15 and 18 and averaged 17 years. Information was also secured from each judge as to his or her birthplace, together with the state of longest residence. The adults were born in 28 different states of the Union and Canada, only one in the state of Florida. More than one adult was born in Georgia, Illinois, Maryland, Massachusetts, Montana, Pennsylvania, South Carolina, and Texas. In most cases the adults indicated that their longest residence was in states other than where they were born, with eight of the 39 having resided longest in Florida. It was interesting to note that 12 of the 23

[1] Acknowledgment is due Mr. G. A. Meckstroth for his assistance with the chemical analyses and the taste testing; Mr. H. L. Schofield, Instructor of Chemistry; and the students of the Orlando, Florida, Senior High School chemistry class for their co-operation.

high school students were born in Florida and that 14 of them had resided there longest.

The basic data are summarized (Table 1); the 12 samples, three from each of four lots, were arranged in random order for tasting.

TABLE 1

Laboratory Determinations and Average Palatability Ratings for 12 Samples of Temple Oranges

Source	Rootstock	Sample No.[1]	Vitamin C	Total solids	Acid	Average palatability grade	
						Students	Adults
			mg./ml.	*pct.*	*pct.*		
Merritt Island	Sour orange	1	.58	12.65	1.11	92	95
		8	.59	12.85	1.10	94	95
		10	.61	13.30	1.09	91	95
	Rough lemon	4	.53	12.20	0.94	93	94
		6	.53	12.10	0.90	92	96
		11	.55	12.60	0.93	92	92
Lake Wales	Sour orange	2	.55	12.25	1.23	86	94
		3	.56	12.10	1.23	88	94
		7	.58	12.65	1.25	87	94
	Rough lemon	5	.51	11.40	1.00	88	95
		9	.50	11.45	0.94	90	93
		12	.51	11.60	0.98	91	93

[1] Number indicates order of tasting.

An analysis of variance was made of laboratory determinations and is summarized (Table 2). With dissolved solids, which indicate sweetness, both location and rootstock differed significantly. Since there was no significant interaction, sample variance functioned as error. Merritt Island fruit scored higher than Lake Wales, and that grown on sour orange stock was higher than that on rough lemon.

TABLE 2

Analysis of Variance of Laboratory Determinations

Source of variation	Degrees of freedom	Mean square for		
		Solids	Acid	Vitamin C
Locations	1	1.51[1]	.0262[1]	.0027[1]
Rootstocks	1	1.65[1]	.1452	.0096[1]
Interaction, R x L	1	0.03	.0056[1]	.0000
Samples within lots	8	0.07	.0004	.0002

[1] Highly significant.

With acidity, interaction is significant and must function as error in the broad sense for rootstock (Table 2). With only one degree of freedom for error this cannot be recognized as significant, in general, although fruit on rough lemon stock is definitely lower in acidity in these particular 12 lots. With rough lemon stock, locality differences are not as clear-cut as with sour orange, leading to a significant interaction.

With vitamin C content, by coincidence, the estimate of interaction was exactly zero. This may happen occasionally with only four classes and a small range of numerical results. Using sample variance as error, both rootstocks and locations showed significant differences, especially rootstocks. Sour orange stock was consistently higher in vitamin C.

Correlations were studied in this material only by preliminary plotting. Vitamin C showed little correlation with ratings or acid, but some association with solids. Acids and solids show little correlation in the small range in this material. Adults' ratings show less correlation with laboratory determinations than the students', whose scores agreed more closely with the content of solids and acid than the adults'. This suggested a keener taste in the younger group.

Next was undertaken analysis of variance of the ratings themselves. The doubt that these are fully suited for analysis of variance has been expressed, but the ratings may well be analyzed for what they can show. All lots of fruit were of very good quality and rated high; most of the ratings were in the range from 80 to 98. Analysis of variance of all ratings is shown first (Table 3).

TABLE 3

Analysis of Variance of Palatability Ratings

Source of variation	Degrees of freedom	Mean square
Between groups	1	2778.0[1]
Tasters within groups	60	159.5[1]
Between locations	1	754.0[1]
Between rootstocks	1	0.5
Interaction, R x L	1	27.5
Samples within lots	8	41.0[2]
Group x location	1	458.0[1]
Group x rootstock	1	187.5[2]
G x L x R	1	70.5[2]
Group x samples within lots	8	50.9[1]
Taster within group x location	60	34.6[1]
T.W.G. x rootstock	60	30.1[1]
T.W.G. x R x L	60	16.0
T.W.G. x sample within lot	480	19.1

[1] Highly significant, one-per cent point. [2] Significant, five-per cent point.

The principles mentioned in the previous article will apply to selection of error variances for different questions. The error for a given comparison must express all the uncontrolled variation affecting that question. For groups, tasters within group will provide error. Questions of difference of tasters will be referred to their interaction with samples as error; differences among samples will be referred to interaction with tasters. Rootstocks should be referred to R x L, and location and R x L to samples, from the standpoint of the present inquiry.

Order of tasting was not considered in analysis; inspection of these data, as well as previous work, indicated that it has no consistent effect with a moderate number of samples.

There is a very marked difference between groups, the students scoring fruit lower. There is also a difference between locations, Merritt Island

scoring higher on the whole. Both groups and tasters showed definite interaction with location and rootstocks, especially groups. This indicates that separate analyses of groups would be informative. The interaction between groups and fruit sources tends to cover up the tendency of each group to score differently.

TABLE 4

Separate Analyses of Variance of Students' and Adults' Ratings

Source of variation	Students		Adults	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Tasters	22	66.7[1]	38	213.2[1]
Locations	1	1136.0[1]	1	75.9
Rootstocks	1	110.0	1	77.5
Rootstock x location	1	97.0	1	1.4
Samples within lots	8	35.9	8	56.1[1]
Taster x location	22	57.9[1]	38	21.1
Taster x rootstock	22	33.8[2]	38	28.0
T x R x L	22	24.8	38	11.0
Taster x samples within lots	176	18.6	304	19.4

[1] Highly significant. [2] Significant.

The separate analyses are shown (Table 4). Here the error for tasters is interaction with samples; sample differences function as error for location and R x L, while R x L is error for rootstock as before. Individual tasters showed definite though not pronounced differences, greater among adults than students. The high school students scored marked differences between locations not scored by adults; these differences are borne out by the laboratory determinations as already mentioned. The students showed a different verdict as to rootstocks than did adults, placing rough lemon higher than sour orange, but the difference was not significant. Students showed more marked individual interactions with location and rootstock than did adults.

DISCUSSION AND CONCLUSIONS

The palatability ratings were put to a severer test here than in the previous work, which dealt with large differences in maturity with advance of season. In the present work differences were small, and adults did not show significant discrimination between lots, but there were moderate differences between judges. High school students showed smaller differences among judges and succeeded in discriminating between lots.

The greater uniformity among high school students' individual total scores might be ascribed to more uniform age and background. The adults showed little preference between lots, while the students were more in agreement with laboratory findings, suggesting keener taste in the younger group. The students were also more severe, giving lower grades. Smoking as a possible explanation of duller taste among adults did not apply here when smoking and non-smoking adult ratings were compared.[2] It should

[2] Their differences are the subject of a proposed article.

be noted that the majority of the adult tasters were men, while the students included both boys and girls.

In the present study there is no real evidence of rootstock difference in palatability but definite evidence of superiority of sour orange to rough lemon in vitamin C and total solids. Certain samples of fruit were somewhat superior to others in chemical constitution and in palatability. This may, however, be due merely to the stage of advancement of the crop or to some grove characteristic rather than to real locational differences.

It may be concluded that rough lemon shows some chemical inferiority to sour orange, which requires careful measurement to detect; that one source was somewhat better than the other; and that high school students judged more severely and apparently discriminated more than adults.

PASTEURIZATION OF LIQUID-EGG PRODUCTS
IV. DESTRUCTION OF COLIFORMS [1]

A. R. WINTER AND G. F. STEWART
Iowa Agricultural Experiment Station, Poultry Husbandry Subsection, Ames, Iowa

AND

MARIAN WILKIN
Ohio State University Research Foundation, Columbus, Ohio

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The coliform group of bacteria, as defined by Breed and Norton (1937) and the American Public Health Association (1941), includes all aerobic and facultative anaerobic, gram-negative bacteria which ferment lactose with gas formation. The two principal species belonging to the group are *Escherichia coli* and *Aerobacter aerogenes*. Griffin and Stuart (1940) have shown that *Escherichia coli* is a normal inhabitant of the intestinal tract and is always found in feces. *Aerobacter aerogenes*, on the other hand, is usually associated with non-fecal material. Garrard (1946) has found both *Escherichia coli* and *Aerobacter aerogenes* in a few shell eggs.

Escherichia coli has long been recognized as an indicator of fecal pollution. Its presence might indicate the presence of enteric pathogens, such as *Eberthella*, *Shigella*, or *Salmonella*, which are also inhabitants of the intestinal tract and found in feces. The presence of coliforms is more easily detected than the presence of enteric pathogens. Consequently the coliform test has been used as a measure for "safe" drinking water by the American Public Health Association (1936); for the production of certified milk by the American Association of Medical Commissioners (1940); and, as a measure of sanitation in various food production processes by Hunter (1939) and Tanner (1944).

Schneiter, Bartram, and Lepper (1943) reported the presence of coliforms in all of eight lots of commercial liquid whole egg examined. Freezing and storage did not kill all of the coliforms. Winter, Greco, and Stewart (1946) reported coliforms in all of 37 samples of commercial liquid whole egg examined. Preliminary studies indicated that coliforms in liquid egg were easily destroyed by pasteurization.

The objectives of this study were (1) to obtain additional data on the occurrence of coliforms in liquid whole egg, (2) to determine the pasteurization temperatures and times required for total destruction of

[1] The subject matter of this paper has been undertaken in co-operation with the Committee on Food Research of the Quartermaster Food & Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the author(s). They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

coliforms in liquid whole egg, and (3) to determine the relationship of the times and temperatures required for the destruction of coliforms to those required for the destruction of *Salmonella*.

EXPERIMENTAL PROCEDURE

Liquid-egg samples were collected, handled, and analyzed for bacterial content according to the method of the American Public Health Association (1941). Standard plate counts were made on Bacto tryptone glucose extract agar and incubated at 32°C.(89.6°F.) for 72 hours. Coliform counts were made on desoxycholate agar according to the method of Leifson (1935) and the Quartermaster Corps, U. S. Army (1944). Egg samples were pasteurized by the procedure described by Winter, Greco, and Stewart (1946).

RESULTS

Thirty-six samples of commercial liquid whole egg were obtained from five egg-breaking plants at different seasons of the year. Twenty-five samples of liquid whole egg were obtained from lots of eggs varying from 10 to 108 dozen broken out in the laboratory. These lots were prepared from cracked, dirty, and Grade C eggs obtained locally. Fifteen samples of commercial frozen egg were collected at four local bakeries. They had been obtained from three different frozen-egg packers. Forty-eight samples of defrosted, commercial whole egg were obtained from four local bakeries. These eggs had been purchased from seven different frozen-egg packers. All of the above samples were analyzed for standard plate and coliform bacteria content; the data are summarized (Table 1).

TABLE 1

Bacterial Content of Fresh, Frozen, and Defrosted Liquid Whole Egg

Kind of sample	Number of samples	Bacteria per ml. (thousands)					
		Standard plate			Coliform		
		Average	High	Low	Average	High	Low
Fresh laboratory	25	359.7	2,950	0.2	10.9	121.5	0.0
Fresh commercial	36	2,718.0	20,200	17.0	61.0	320.0	0.3
Frozen commercial	15	3,304.5	28,500	17.5	6.0	30.0	0.3
Defrosted commercial	58	15,813.7	138,000	235.0	663.0	5,340.0	0.4

A total of 134 samples of liquid whole egg were analyzed for standard plate and coliform bacteria. Commercial, fresh whole-egg samples varied greatly in bacterial content. This was true of samples obtained at the same plant as well as of those from different plants. The bacterial content of samples obtained under commercial plant conditions was much higher than that of samples obtained under laboratory conditions. This was true in spite of the fact that low-grade, dirty, and cracked eggs were used in their preparation.

The commercial liquid whole egg sampled in the frozen state had a high bacterial content. All the samples contained coliforms.

Defrosted, commercial, liquid whole-egg samples contained far more bacteria than either the unfrozen or frozen samples.

All commercial samples, whether fresh, frozen, or defrosted, contained coliforms. In general, the number appeared to be correlated with the number of standard-plate-count bacteria present.

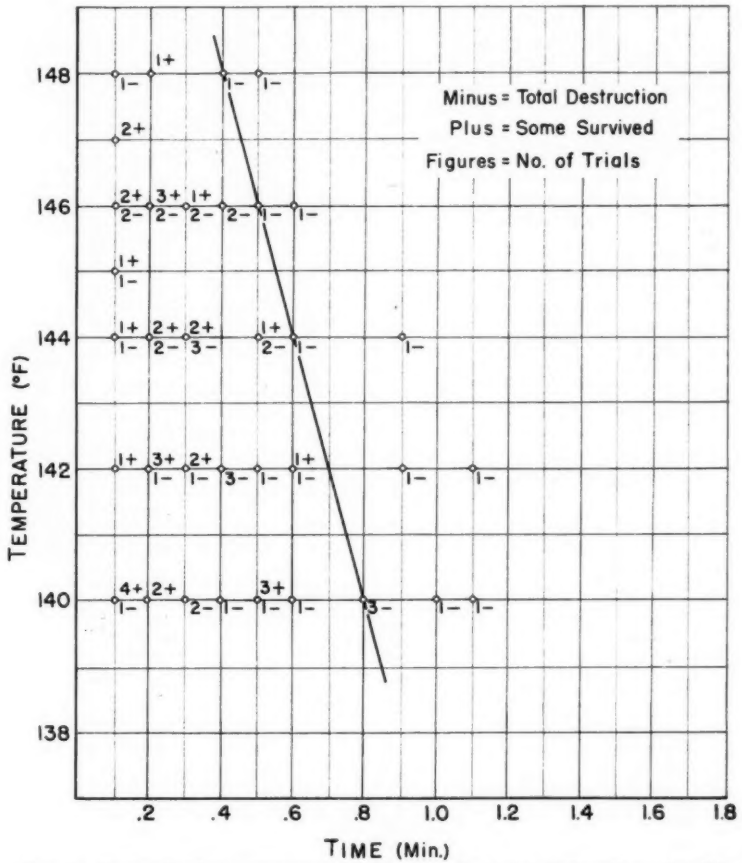

FIG. 1. Pasteurization temperatures and times for total destruction of coliforms in fresh liquid whole egg.

Pasteurization Tests: Eighteen samples of freshly broken, commercial, liquid whole egg were obtained from five egg-breaking plants for pasteurization. The coliform bacteria in the samples at time of pasteurization ranged from 235 to 1,035,000 per milliliter. The samples were pasteurized at different temperatures and times as shown (Table 2 and Fig. 1). The coliforms were killed easily at 63.3°C.(146°F.) in 0.4 minute, 62.2°C. (144°F.) in 0.6 minute, 61.1°C.(142°F.) in 0.7 minute, and 62°C.(140°F.) in 0.8 minute.

TABLE 2

Destruction of Coliform Bacteria in Commercial Liquid Whole Egg by Pasteurization

Pasteurization		Coliforms per ml.	
Temperature	Time	Before pasteurization	After pasteurization
°F.	min.		
	0.1	429,500	0
148	0.2	1,035,000	595
	0.5	.....	0
	0.4	7,000	0
147	0.1	425,000	335
	0.1	129,500	30
	0.1	320,000	40
	0.2	.....	0
	0.1	429,500	0
	0.1	235	0
	0.1	11,500	5
	0.6	.....	0
	0.2	30,500	105
146	0.3	.....	0
	0.2	2,300	0
	0.2	43,500	130
	0.3	.....	0
	0.2	1,035,000	625
	0.5	.....	0
	0.3	4,500	5
	0.4	.....	0
	0.4	7,000	0
145	0.1	1,350	0
	0.1	8,950	360
	0.1	235	0
	0.1	429,500	20
	0.5	.....	0
	0.2	280	0
	0.2	2,300	0
	0.2	320,000	5
	0.3	.....	0
144	0.2	11,500	40
	0.9	.....	0
	0.3	7,000	0
	0.3	4,600	0
	0.3	30,500	95
	0.6	.....	0
	0.3	43,500	10
	0.5	.....	0
	0.5	1,035,000	5

Pasteurization		Coliforms per ml.	
Temperature	Time	Before pasteurization	After pasteurization
°F.	min.		
	0.1	429,500	35
	0.6	.....	0
	0.2	11,500	335
	1.1	.....	0
	0.2	235	5
	0.4	.....	0
	0.2	2,300	0
	0.2	43,500	660
142	0.4	.....	0
	0.3	7,000	0
	0.3	4,600	55
	0.4	.....	0
	0.3	320,000	5
	0.5	.....	0
	0.6	30,500	40
	0.9	.....	0
	0.1	1,350	0
	0.1	3,350	25
	0.3	.....	0
	0.1	8,950	1,075
	0.5	.....	0
	0.1	1,000	235
	1.0	.....	0
	0.1	429,500	1,025
	0.6	.....	0
140	0.2	6,000	90
	0.2	11,500	450
	1.1	.....	0
	0.3	235	0
	0.4	7,000	0
	0.5	4,600	5
	0.8	.....	0
	0.5	30,500	200
	0.8	.....	0
	0.5	320,000	20
	0.8	.....	0

Sixteen samples of commercial, defrosted, liquid whole egg were obtained from four local bakeries for pasteurization studies. The lots had been packed at seven different egg-breaking and freezing plants. The coliform count at time of pasteurization ranged from 1,800 to 15,000,000 per milliliter; results are shown (Table 3 and Fig. 2). Three of the

TABLE 3

Destruction of Coliform Bacteria in Commercial, Defrosted Liquid Whole Egg by Pasteurization

Pasteurization		Coliforms per ml.	
Temperature	Time	Before pasteurization	After pasteurization
°F.	*min.*		
148	0.5	79,500	0
	0.6	2,550,000	0
147	0.8	56,500	0
	0.2	12,500	0
	0.2	4,200	0
	0.2	1,800	0
	0.2	5,340,000	5
146	0.3	.....	0
	0.4	15,000,000	10
	0.5	.....	0
	0.4	530,000	50
	0.5	.....	0
	0.7	2,550,000	0
	0.2	4,200	0
	0.2	1,800	0
	0.2	12,100	15
	0.3	.....	0
	0.2	5,340,000	15
	0.3	.....	0
	0.4	15,000,000	5
144	0.6	.....	0
	0.4	6,750	0
	0.6	4,100	0
	0.6	530,000	680
	0.8	.....	0
	0.7	2,550,000	10
	0.8	.....	0
	1.0	56,500	0
	1.4	405,000	0
	1.8	55,000	0

Pasteurization		Coliforms per ml.	
Temperature	Time	Before pasteurization	After pasteurization
°F.	*min.*		
	0.2	4,200	0
	0.2	1,800	0
	0.4	6,750	0
	0.4	12,100	5
	0.6	.....	0
	0.4	5,340,000	5
142	0.6	.....	0
	0.6	4,100	0
	0.6	15,000,000	5
	0.8	.....	0
	0.7	2,550,000	15
	1.5	.....	0
	1.1	530,000	70
	1.4	55,000	0
	0.3	1,800	0
	0.4	4,200	0
	0.4	6,750	0
	0.5	5,340,000	0
	0.5	12,100	40
	0.7	.....	0
	0.6	4,100	0
140	0.8	56,500	100
	1.2	15,000,000	5
	1.9	.....	0
	1.4	2,550,000	0
	1.5	55,000	0
	1.7	6,750	0
	1.8	530,000	145
	2.2	.....	0
	0.4	6,750	0
138	0.6	4,100	0
	1.5	55,000	0
	1.8	8,750	0

samples contained coliforms that were more heat resistant than those found in the unfrozen samples (Figs. 1 and 2). One lot of egg contained a coliform that was not destroyed at any of the temperatures and times used (Tables 2 and 3). Identification studies showed it to be *Aerobacter aerogenes*. With the exception of this organism in one sample, the coliforms were killed at 146°F. in 0.5 minute, 144°F. in 1.0 minute, 142°F. in 1.5 minutes, and 140°F. in 2.5 minutes.

DISCUSSION

The widespread occurrence of coliform bacteria in liquid, frozen, and defrosted whole egg has been reported by several investigators, including Redfield (1920), Brownlee and James (1939), and Schneiter, Bartram, and Lepper (1943). The data presented here is in agreement with that

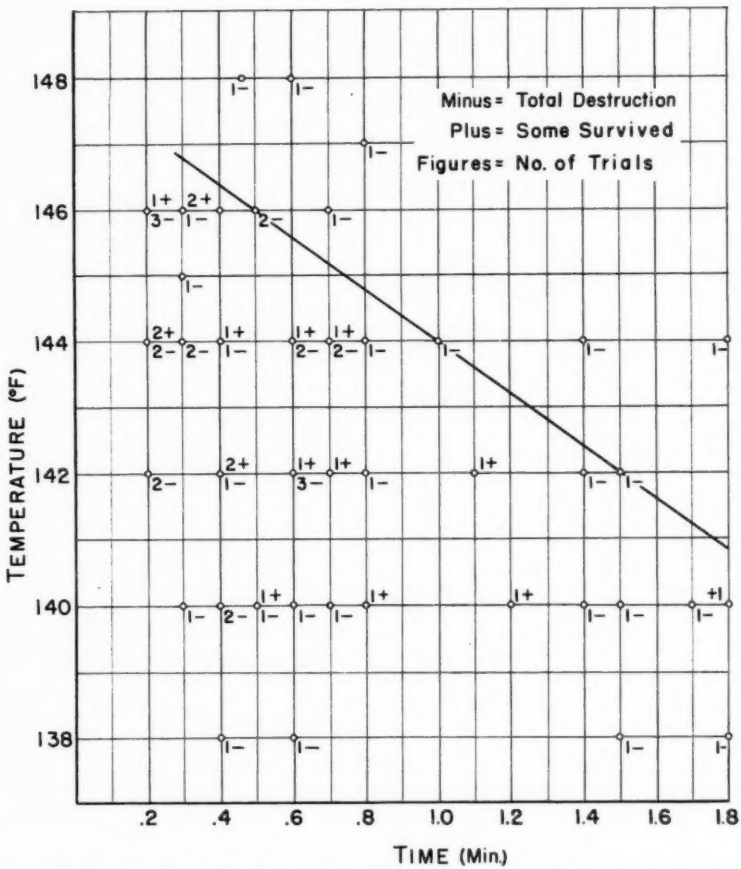

FIG. 2. Pasteurization temperatures and times for total destruction of coliforms in defrosted commercial liquid whole egg.

reported by Redfield (1920) in that the bacterial content of liquid egg is much greater when eggs are broken under commercial plant conditions than when broken under laboratory conditions. This would indicate that the sources of most of the bacteria appearing in liquid egg are the shell, equipment, handlers, etc., and not the egg contents, and/or that the conditions under which the product is handled permit gross multiplication to occur.

The wide variation in heat resistance of the coliforms found in liquid, frozen, and defrosted whole egg was anticipated. Heat-resistant coliforms have been found occasionally in milk products by Graham (1936), Leahy (1938), Long, Hedrick, and Hammer (1944), Smith (1944), and Tiedeman and Smith (1945). Long, Hedrick, and Hammer (1944) cautioned against condemning a product as having been improperly pasteurized or re-contaminated until the coliforms found have been tested for heat resistance. It would also be desirable to identify the coliform since *E. coli* is of fecal origin while *Aerobacter aerogenes* is generally of non-fecal origin. De Bard (1925) has also shown that the colon type is less heat resistant than the aerogenes type.

In general, the greater the number of total coliforms present the higher the temperature or the longer the time or both that was required for their total destruction. This is in agreement with work reported by Bendixen, Blink, Drummond, Leroy, and Wilson (1937).

Winter, Stewart, McFarlane, and Solowey (1946) found that the species of *Salmonella* most frequently found in liquid whole egg were destroyed by pasteurization at 146°F. for 0.8 minute, 144°F. for 1.2 minutes, 142°F. for two minutes, and 140°F. for 2.6 minutes. Therefore, within the limit of samples studied thus far, pasteurization of liquid whole egg at 146°F. for one minute, 144°F. for 1.5 minutes, 142°F. for 2 minutes, and 140°F. for 2.6 minutes will destroy all coliforms and *Salmonella.*

SUMMARY

Coliform bacteria were found in all of the 134 samples of commercial, liquid, frozen and defrosted whole egg examined.

Pasteurization of unfrozen and defrosted, liquid whole egg at 146°F. for 0.5 minute, 144°F. for 1.0 minute, 142°F. for 1.5 minutes, and 140°F. for 2.5 minutes resulted in total destruction of coliforms in all but one of the samples tested.

Coliform bacteria found in unfrozen, liquid whole-egg samples were killed more easily by pasteurization than those found in defrosted, liquid whole egg.

The coliform test may be used as a measure of egg-breaking-plant sanitation. The coliform test is not a reliable index of the efficiency of pasteurization, since the coliforms in some samples may be destroyed more easily than types of *Salmonella.*

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PACKAGING DEHYDRATED MEAT[1]

R. A. WHITMORE
Agricultural Experiment Station, Rio Piedras, Puerto Rico

DAVID SELIGSON,[2] H. R. KRAYBILL
American Meat Institute, Chicago, Illinois

AND

B. H. WEBB
Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture

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Early in 1942 it became apparent that a serious tin shortage was imminent in the United States. The consumption of tin in packaging foods for the Army and for Lend Lease was increasing rapidly. Therefore, as a part of the comprehensive research program on dehydrated meat, the Agricultural Research Administration of the United States Department of Agriculture in co-operation with the American Meat Institute and the University of Chicago, instituted a study of the packaging and keeping quality of that product. One purpose of the project was to discover, or devise, containers for dehydrated meat which would require no tin or substantially less tin than the usual tin can.

Inasmuch as information was not available which would permit evaluation of a package for dehydrated meat in terms of physical constants, the method used for testing packages was to pack a number of trial containers with dehydrated meat, submit the packages to an accelerated test in a controlled storage room or in an oven, and at the end of various time intervals test the contents organoleptically and chemically.

EXPERIMENTAL PROCEDURE

A study was made of many types of metallic and nonmetallic packages. The metallic cans were of three types: (1) hot dipped tin plate, the type usually used for food products; (2) fused electrolytic tin plate, requiring approximately two-fifths as much tin as the first type; and (3) "Bonderized" metal cans requiring no tin. The term "Bonderized" refers to a proprietary phosphate treatment for sheet steel that prepares the metal for a resinous coating. Eighteen test packs of approximately 100 cans each were made with metallic containers. Dehydrated beef was packed in air, in nitrogen, and in vacuum in each of the three types of containers listed above; and dehydrated pork was also packed in air, in nitrogen, and in vacuum in each of the three types of containers.

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[1] This paper is one in a series, appearing in *Food Research*, which deals with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture and certain co-operators.

[2] Formerly of the Agricultural Research Administration, U. S. Department of Agriculture.

A representative group of paper and metal-foil packages was studied with a view to determining their suitability as substitutes for cans. The materials, laminating agents, and sealing compounds varied with different makes of bags which were of the envelope type—flat bags which when opened become containers with rectangular sides. These bags were approximately five and one-half by eight and one-half inches in size and, when filled, were fitted into cartons. Some bags had stitched closures; some were wax dipped; many were fabricated with special laminating agents and heat-sealing compounds.

The bags were constructed according to one of three plans: (1) a cylinder formed by an overlapped, heat-sealed side seam was heat-sealed across the bottom, the resultant package having one bottom seam and one overlapped side seam; (2) a sheet of the package film was doubled and the two sides heat-sealed, the resultant package having no bottom seam and two side seams; (3) two sheets of the package film were placed face to face and heat-sealed across the bottom and sides, the resultant package having one bottom seam and two side seams. These envelopes were shaped over a wooden mandrel, placed in a supporting form, and packed firmly with dehydrated pork. The top was then folded over and heat-sealed, care being taken to keep the volume of free air at a minimum.

A brief description of some of the paper and metal-foil packages which were studied follows:

A. 55-pound laminated glassine; thermoseal tops and bottoms.
B. 25-pound glassine, 300 M.S.A.T. (moisture-proof, heat-sealing, anchored, transparent) cellophane; two side seals. (The term "anchored" signifies a process by which the heat-sealing material is firmly fastened to the cellophane.)
C. 45-pound opaque glassine and 300 M.S.A.T. cellophane laminated together with the cellophane inside; two side seals.
D. A double thickness of 450 M.S.A.T. cellophane laminated together; two side seals.
E. Asphalt-impregnated kraft, 0.0005-inch lead foil, and 300 M.S.A.T. cellophane laminated together with the cellophane inside; two side seals.

The dehydrated pork was prepared as follows:

Fresh lean pork was cut into one-inch strips and cooked in a steam-jacketed kettle with mechanical stirring for 30 minutes at a maximum temperature of 65.6° C. (150° F.). The aqueous and fatty liquor that separated during cooking was drained and concentrated *in vacuo* to be re-added to the finished product. The cooked meat was ground through a 1/8-inch plate and dehydrated in a machine of the type designated as air-rotary Dehydrator B in *Food Research* (1945) **10**, 388-390. The meat reached an internal temperature of 54.4 to 57.2° C. (130 to 135° F.) in the dehydrator. The dehydrated meat was placed in a mixer and salt was added to bring the salt content to three per cent. Some dehydrated pork packed in Bags A to E contained no salt. Fat and the concentrated aqueous liquor, separated during cooking, was then added to bring the fat content to 40 per cent and the moisture content to approximately 10 per

cent. The meat was then well mixed and placed in 75-pound metal drums which were kept under refrigeration until the packages were filled.

The dehydrated beef was prepared in a manner similar to that used for the dehydrated pork, except that the beef was cooked for 20 minutes at a maximum temperature of 73.9°C.(165°F.). The liquid that separated during the first stage of the cooking was reabsorbed during the final stage so that no free liquor was present, nor was any added after dehydration. During dehydration the beef reached an internal temperature of 60 to 62.8°C.(140 to 145°F.) and was dehydrated to a final moisture content of 7.9 per cent. A proximate analysis gave the following:

	Beef	Pork
	pct.	*pct.*
Fat	26.2	35.1
Protein	60.4	51.5
Moisture	7.9	10.5
Salt	3.5	2.5

Part of the packages were stored in the laboratories of the American Meat Institute in a refrigerator at 3.3 to 4.4°C.(38 to 40°F.), at room temperature [21.1 to 32.2° C.(70 to 90° F.)], and in an oven at 57.2° C. (135°F.). The last temperature, 135°F., was selected for two reasons: (1) it served as an accelerated test, and (2) it was used to equal, or surpass, the worst possible storage conditions likely to be encountered. Another group of packages was stored in laboratories of the Agricultural Research Administration at temperatures of —17.8, 10, 21.1, and 43.3°C. (0, 50, 70, and 110°F.). All temperature rooms in this latter group, except that at 0°F., were maintained at a constant relative humidity of 80 per cent.

A number of packages of all kinds were stored at alternated temperatures, a method of testing known as cycling. Some were cycled between 0 and 110°F. and others between 50 and 110°F. Unless otherwise stated all packages were alternated on a 24-hour cycle. The relative humidity on the 50 to 110°F. cycle was 80 per cent.

At the conclusion of suitable periods of storage, samples of each kind of meat from each of the test packs from each of the storage temperatures were opened and the extracted fat was tested for peroxide value and free fatty acids. The peroxide value was determined by extracting a sample of meat with chloroform, filtering it into a tared beaker, weight W_1, and weighing the beaker plus extract, weight W_2. An aliquot, approximately three-fourths of the extract, was poured from the beaker into a 400-ml. Erlenmeyer flask, the beaker plus the remainder of the extract reweighed, weight W_3, and set on a hot plate for evaporation of the chloroform. A small quantity, three-fourths to one ml., of saturated potassium iodide solution was added to the extract in the Erlenmeyer flask; the contents of the flask were well mixed and allowed to stand three to five minutes. The weight of the extract in the flask was determined, W_2-W_3, and a volume of glacial acetic acid equal in milliliters to the weight of the extract in grams was added to the flask and the contents mixed. A volume of distilled

water estimated equal to the volume of the fat-chloroform-acetic acid mixture was added to the flask, three to five ml. of starch indicator was added, and the iodine liberated by oxidation of the iodide determined by titration with N/10 thiosulfate. The beaker containing the remainder of the extract was weighed after evaporation of the chloroform, weight W_4, and the amount of fat in the beaker determined, W_4-W_1. The amount of fat present in the extract in the Erlenmeyer flask was calculated, $\frac{(W_4-W_1)(W_2-W_3)}{(W_3-W_1)}$. The peroxide value, P.V., was determined by multiplying milliliters of thiosulfate solution used by the normality of the solution by 1,000 and dividing the product thus obtained by the weight of the fat in grams.

The free fatty acid value, expressed as percentage of fat hydrolyzed to free fatty acids calculated on the basis of oleic acid, was determined by adding 20 milliliters of neutral ethanol containing phenolphthalein to an aliquot of the chloroform-free fat sample used in the peroxide determination. The alcoholic solution was heated to boiling on a hot plate and titrated to a definite pink color with N/10 sodium hydroxide. The free fatty acid value was determined by multiplying the milliliters of hydroxide solution by the normality of the solution by 28.2 and dividing the product so obtained by the weight of the sample.

Samples were also tested organoleptically, in some cases for evidence of protein breakdown, and in a few cases test meat loaves were made.

RESULTS

The results of tests for peroxide value and free fatty acids made in Bonderized cans are given (Tables 1 and 2). The same general behavior was exhibited by each series of samples. The peroxide value increased to a maximum at a rate correlated with the temperature of storage, then decreased slowly to zero in those samples stored at high temperatures. The free fatty acid content slowly increased with a slowly increasing rate in the samples stored at the higher temperatures. No significant difference was found between the three types of metal containers nor was there a significant difference between the three types of packing in the cans. The peroxide value of those samples packed in air reached a higher maximum value than in the samples packed in nitrogen or in vacuum, but the limited amount of oxygen available in the sealed can prevented oxidative changes from affecting the quality of the meat. In none of the samples was there any evidence, organoleptic or chemical, of development of rancidity during one year of storage. No significant difference from the values given (Tables 1 and 2) was found for other types of metal cans; therefore, the data for the other storage tests are not given.

To study the relationship between oxygen absorption and peroxide value in dehydrated meat packed in air, dehydrated beef and pork were air-packed in No. 2 tin cans with from three-fourths to one inch of head space. The cans were stored at 23.9 to 26.7°C.(75 to 80°F.), room temperature, and at intervals were sampled for oxygen and carbon dioxide content

TABLE 1

Peroxide Value of Dehydrated Beef and Pork Packed in "Bonderized" Cans as Affected by Storage Time and Temperature

Storage time (weeks)	135°F.			110°F.			70-90°F.			36-38°F.		
	A[1]	N[2]	V[3]	A	N	V	A	N	V	A	N	V
Beef												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	1.3	2.1	0.9	....	....	....	....	....	....	....	....	....
4	0.9	1.5	1.0	3.4	4.7	3.4	4.3	4.1	3.4	3.8	5.0	3.2
6	1.7	2.4	2.4	....	....	....	....	....	....	....	....	....
8	1.7	1.2	1.7	....	....	....	....	....	....	....	....	....
12	0.8	0.5	0.5	1.4	0.3	0.9	0.5	0.6	2.5	2.1	1.0	1.3
24	1.9	0.9	0.8	1.4	1.0	2.1	0.7	1.7	1.4	1.6	1.3	1.4
30	0.4	0.7	0.5	....	....	....	....	....	....	....	....	....
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pork												
0	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
2	1.5	1.4	1.2	....	....	....	....	....	....	....	....	....
4	0.6	0.6	0.5	1.8	1.7	2.4	1.4	0.3	0.2	3.0	2.4	0.9
8	0.8	1.1	1.3	....	....	....	....	....	....	....	....	....
12	0.5	0.5	0.5	0.5	0.6	0.5	1.3	1.4	2.4	2.9	1.8	2.3
24	0.3	0.3	0.9	0.5	0.5	0.6	1.0	1.0	1.0	1.0	1.3	0.9
30	0.2	0.0	0.1	....	....	....	....	....	....	....	....	....
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[1] Air pack. [2] Nitrogen pack. [3] Vacuum pack.

TABLE 2

Percentage of Free Fatty Acid in Dehydrated Beef and Pork Packed in "Bonderized" Cans as Affected by Storage Time and Temperature

Storage time (weeks)	135°F.			110°F.			70-90°F.			36-38°F.		
	A[1]	N[2]	V[3]	A	N	V	A	N	V	A	N	V
Beef												
0	0.5	0.8	0.8	0.5	0.8	0.8	0.5	0.8	0.8	0.5	0.8	0.8
4	0.9	1.5	0.9	1.2	1.5	1.5	1.3	1.3	1.2	1.1	1.4	1.6
12	2.0	1.7	2.1	1.0	0.9	1.2	0.8	0.8	0.9	0.9	0.7	1.0
17	3.2	2.7	2.7	....	....	....	....	....	....	....	....	....
24	3.8	3.3	3.7	2.8	1.6	2.9	0.8	0.6	0.8	0.6	0.6	0.7
30	5.1	5.2	4.1	....	....	....	....	....	....	....	....	....
40	7.8	6.9	6.6	3.6	2.2	5.4	0.9	0.9	0.9	0.6	0.6	0.6
Pork												
0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4	0.4	0.7	0.5	1.3	1.2	1.1	0.6	0.6	....	0.5	0.4	0.8
12	1.3	0.9	1.6	0.3	0.3	0.5	0.4	0.3	0.3	0.3	0.4	0.4
17	0.5	1.0	0.3	....	....	....	....	....	....	....	....	....
24	2.4	2.0	2.4	1.1	0.5	1.0	0.4	0.2	0.2	0.3	0.3	0.4
30	2.9	2.9	3.0	....	....	....	....	....	....	....	....	....
40	5.0	4.6	4.6	2.1	1.4	3.2	0.5	0.5	0.6	0.3	0.4	0.5

[1] Air pack. [2] Nitrogen pack. [3] Vacuum pack.

of the residual gas and for peroxide number of the meat; the results are shown graphically (Fig. 1). In both dehydrated beef and pork, oxygen absorption showed no induction period; in a relatively short time, four weeks in the dehydrated pork and nine weeks in the beef, the oxygen con-

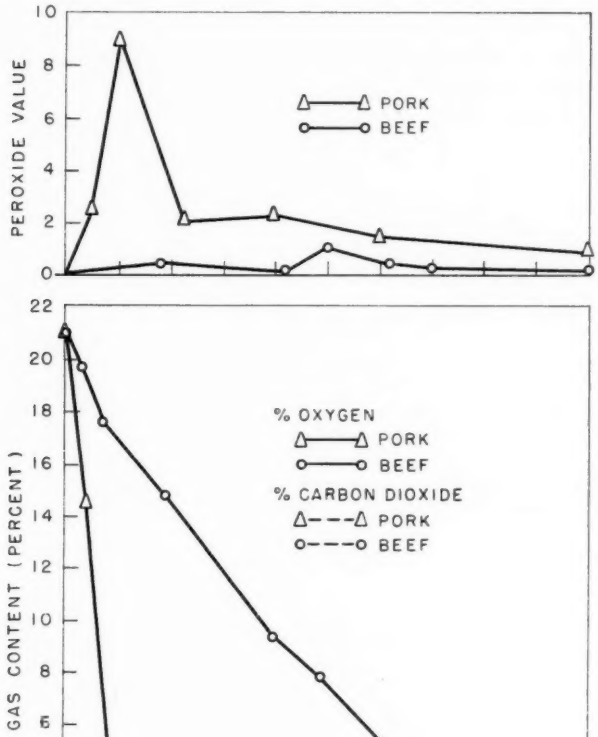

FIG. 1. Changes in composition of the residual gas and in peroxide value of dehydrated meat during storage in cans at room temperature.

tent of the gas in the head space of the can decreased to approximately 0.4 per cent; and at the end of 10 weeks at room temperature the peroxide value of both kinds of meat was below 1.0. Both the beef and pork were palatable throughout the test and no trace of rancidity was noted in taste tests. The head space in the sample cans was approximately four times that in commercially packed samples.

The envelope-type packages in the first group tested were uniformly bad. The failures were from two causes: (1) the seals were not tight; and (2) the laminating agent used in preparing the film was fat soluble and the lamina separated. Envelopes made so that the meat did not come in contact with the edges of the film eliminated the latter difficulty.

After many modifications of laminating agents and heat-sealing methods, several envelope-type packages were obtained which were fat-tight at elevated temperatures for several months. The comparative performance of a representative group of these bags is shown (Table 3). A control sample was packaged in tin. Where the packages did not fail, the results of the bag tests compared favorably with those obtained with cans. Low peroxide and acidity values did not necessarily indicate that deterioration in the package had not occurred.

Metal-foil package E was superior to the other bags but even this container eventually failed in the severe cycling tests 50-110°F. at 80 per cent relative humidity, as shown by the data (Table 3). A number of these E containers packed with dehydrated pork were placed at 110°F. for 12 hours; at room temperature (about 80°F.) for eight hours; at 0°F. for 12 hours; and at room temperature for eight hours. This cycle was repeated 30 times. The first package failed in 10 cycles and the majority had failed by the end of 30 cycles. Apparently the shearing stress set up between the lamina by the differing degrees of expansion on temperature changes caused the laminations to separate and finally tear. The value of a cycling test as a rapid means of comparing different paper containers should be emphasized.

The effect of wax dipping was tried, using a relatively low-melting-point wax which was somewhat plastic at room temperature and a high-melting-point, micro-crystalline wax which was quite hard at room temperature. Under the storage conditions used for tests, this wax coat over the exterior carton was not beneficial. If the inner cellophane bag was fat-tight, the wax coat was unnecessary; and if the inner cellophane bag was not fat-tight, the solvent action of the fat, in liquid state at the temperatures used for tests, rapidly destroyed the protective effect of the wax coat. Under conditions of high-humidity storage, coating the outer carton with wax greatly reduced the disintegrating effect of the high humidity on the outer cardboard carton.

The envelope-type package was found to be relatively nonresistant to mechanical shock. This was especially true of individual packages. The experience of packaging specialists, however, indicates that a number of small packages properly packed in a correctly designed shipping case can withstand rather rough handling.

Tests were conducted to determine the lead content of meat samples shipped and held in storage in the "E" or lead-foil bags. Twelve packages of the dehydrated pork were prepared with Package E. Twelve lined packages were prepared in the same way except Bag A was used as an inner liner for the lead-foil bag. The packaged meat was shipped by express from Washington, D. C., to Albany, California, and back. The contents of one-third of the packages were analyzed at once for lead; the

TABLE 3

Effect of Storage at 80 Per Cent Relative Humidity, at Different Temperatures, and in Various Packages Upon Properties of Dehydrated Pork[1]

Container	Time of storage	Moisture content after storage at different temperatures				Free fatty acid content after storage at different temperatures				Peroxide value after storage at different temperatures			
		0°F.	70°F.	110°F.	50-110°F.	0°F.	70°F.	110°F.	50-110°F.	0°F.	70°F.	110°F.	50-110°F.
	wk.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*				
Cans (air pack)	0	9.01	9.01	9.01	9.01	0.6	0.6	0.6	0.6	0.1	0.1	0.1	0.1
	8	9.32	9.14	9.03	9.31	0.6	0.6	1.1	0.8	0.3	0.2	0.0	0.0
	24	9.22	9.15	9.08	9.18	0.7	0.9	2.2	1.3	1.1	0.1	0.0	0.0
	40	9.40	9.11	9.00	9.00	0.6	1.1	3.2	1.8	0.8	0.0	0.0	0.0
A[2] glassine bag	0	9.01	9.01	9.01	9.01	0.6	0.6	0.6	0.6	0.1	0.1	0.1	0.1
	8	9.15	9.20	9.55	13.47	0.6	0.8	1.0	1.3	0.3	13.0	28.0	107.8
	24	8.61	9.81	10.04	21.86	0.6	3.2	3.3	8.5	1.0	118.3	1.5	0.0
	40	9.22	10.94	10.99	23.74	0.7	15.7	7.8	22.1	1.0	58.9	0.0	0.0
C[2] cellophane bag	0	9.01	9.01	9.01	9.01	0.6	0.6	0.6	0.6	0.1	0.1	0.1	0.1
	8	9.16	9.29	9.47	10.03	0.6	0.7	1.0	0.8	0.4	0.4	0.0	0.0
	24	9.19	9.26	9.72	11.61	0.7	0.8	1.7	1.8	0.9	33.6	1.3	75.6
	40	9.57	9.97	9.96	12.70	0.7	3.3	3.2	1.7	0.9	107.7	0.0	0.0
E[2] metal foil bag	0	9.01	9.01	9.01	9.01	0.6	0.6	0.6	0.6	0.1	0.1	0.1	0.1
	8	8.88	8.99	8.72	8.12	0.6	0.7	1.1	0.9	0.8	0.0	0.0	0.0
	24	8.51	8.62	8.82	10.50	0.6	0.8	2.1	1.4	1.1	0.1	0.0	0.0
	40	9.55	9.06	9.61	11.27	0.7	1.1	3.3	1.8	0.9	0.1	0.0	0.0

[1] There was no humidity control at 0°F. [2] Containers described on page 20.

contents of the remaining packages were analyzed after periods of storage at 110°F. up to four months after return of the shipment.

Spectrographic studies to determine lead contamination in the meat samples were made.[3] The spectrum of the ashed package material showed that lead, tin, and aluminum were constituents. A sample of dried meat which had not been packed was used as a control. A series of lead standards was also prepared, using the control sample as the base material.

A non-recording densitometer was used to measure the density of characteristic spectral lines. Upon comparison of the densitometer readings for the various samples studied, it was found that no lead contamination from the package had taken place. The readings for the control, "lined," and "plain" samples were of the same order of intensity. All of the lead-standard mixtures containing known amounts of added lead showed higher density readings. It was concluded that no contamination of these meat samples occurred from Package E either with or without an inner liner.

Both dehydrated beef and pork, packed in air, vacuum, or nitrogen and stored at 100°F. or above, developed a marked red-brown color in two weeks or so at 135°F. and in four to six weeks at 110°F. This color seemed to be due to the effect of heat on the nonfat constituents as defatted meat developed the same color in air, nitrogen, carbon dioxide, or in vacuum. Spectrophotometric reflection curves of the red meat showed a general absorption and no evidence that the red color was due to heme pigment or heme-pigment products.

Taste tests were made of samples prepared by cooking in a boiling-water bath 20 grams of dehydrated meat plus 30 grams of water for 30 minutes and scoring samples on the basis of flavor and texture. The tests indicated that in two weeks of storage at 135°F. dehydrated meat developed a marked "cured" flavor which many people preferred to freshly dehydrated meat. This cured flavor slowly changed to an unpalatable "scorched" flavor during six months of storage. Samples stored at 110°F. developed a similar "cured" flavor during six to eight weeks of storage. This flavor was retained with little change during one year of storage, at the end of which the 110°F. samples were still palatable. Samples stored at room temperature (about 80°F.) underwent relatively little change. The original light color slowly changed to yellow and the flavor slowly changed from that of freshly dehydrated meat to an intermediate flavor between fresh and cured meat. Samples stored under refrigeration retained their original flavor and color, measured organoleptically, for a year of storage.

SUMMARY AND CONCLUSIONS

Dehydrated beef and pork were packed in air, in vacuum, and in nitrogen in hot-dipped tin-plate cans, fused electrolytic plate cans, and "Bonderized" cans. Samples were stored at 135°F., 110°F., 70 to 90°F. (room temperature), and at 36 to 38°F.

The development of peroxides and of free fatty acid in the meat was determined after varying lengths of storage. No significant difference was

[3] Spectrographic studies were made by R. E. Davis and Harry Bastron, Bureau of Animal Industry.

found between the air, nitrogen, or vacuum packs or between the samples in the different kinds of cans. The peroxide value reached a maximum at a rate determined by the storage temperature after which it declined slowly to zero. The free fatty acid content increased slowly but not significantly in one year of storage.

Palatability tests indicate that dehydrated beef and pork will remain edible for six months packed in air, in nitrogen, or in vacuum in metal cans at 135°F. Samples were palatable at the end of one year of storage at 110°F., room temperature and 36 to 38°F.

A number of nonmetallic and metal-foil, envelope-type packages were tested as containers for dehydrated pork. The chief cause of failure in packages of this type was leakage through the heat-sealed closures. When these packages were properly sealed and when the sealing compound and laminating agent were not fat soluble, the packages were suitable as containers for dehydrated pork for periods of about one year at room temperature.

Package E made of metal foil laminated to cellophane was the best of the bag-type containers which were studied. Under conditions of storage and shipping in which severe abuse and mechanical shock were avoided this package was very satisfactory as a container for dehydrated pork.

The changes in the peroxide value, free fatty acid content, and palatability of dehydrated meat packaged in the structurally sound, envelope-type bags were similar to the changes which occurred in the same kind of meat packaged in cans.

PHYSIOLOGICAL ACTION OF SODIUM CARBOXYMETHYLCELLULOSE ON LABORATORY ANIMALS AND HUMANS

HERMAN A. SHELANSKI AND ARNOLD M. CLARK
Smyth Laboratories, Philadelphia, Pennsylvania

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Sodium carboxymethylcellulose is the sodium salt of carboxymethylcellulose or cellulose glycolic acid. It is a white, fluffy, hygroscopic solid soluble in water at all temperatures. It has been found to be a useful stabilizer for emulsions, one-half to one per cent being sufficient to stabilize water and a wide range of oils. In aqueous solution it forms a clear, colorless, viscous fluid at three per cent concentration and a gelatinous fluid at five per cent concentration. Concentrations above five per cent will form a gel. Chemical analysis of the product studied in the following experiments is shown (Table 1).

TABLE 1

Chemical Analysis[1] of Sodium Carboxymethylcellulose

Ash	18.8 per cent
Chlorine (Parr bomb)	0.2 per cent
Sodium	7.8 per cent
Carbon	39.0 per cent
Hydrogen	5.2 per cent
Nickel	3 parts per million
Copper	8 parts per million
Lead	7 parts per million

[1] Chemical analysis—Personal communication, W. W. Becker and G. E. Osborn, Hercules Powder Experiment Station.

Brown and Houghton (1941), in considering sodium carboxymethylcellulose, conclude that it is harmless physiologically, that the chemical constitution suggests that it is innocuous, and that feeding tests on animals in no way affected the health of the animals. Post-mortem examinations of the vital organs showed them to be normal in every way. These authors also conclude that sodium carboxymethylcellulose may be used in preparations for internal consumption as well as in toilet preparations for external application.

Werle (1941) also showed that sodium carboxymethylcellulose was harmless physiologically when fed to rats and dogs for a two-month period.

Rowe, Spencer, Adams, and Irish (1944) have shown in their work with rats that sodium carboxymethylcellulose is harmless physiologically and that there is no evidence why these materials should not be incorporated into food for human consumption.

The following experiments corroborate the evidence presented in the above studies.

ACUTE ORAL-TOXICITY STUDIES

Acute oral-toxicity studies were performed on white rats and guinea pigs which were fed varying dosages of sodium carboxymethylcellulose

suspended in olive oil in the ratio of one gram of the salt to two milliliters of olive oil. The suspension was introduced directly into the stomach of the test animal by means of a stomach tube. The following results were obtained:

WHITE RATS	GUINEA PIGS
L.D. 0—20 grams per kilogram	L.D. 0—10 grams per kilogram
L.D. 50—27 grams per kilogram	L.D. 50—16 grams per kilogram
L.D. 100—40 grams per kilogram	L.D. 100 (Higher dosage was not advisable owing to death caused by mechanical injury and to shock from method of administration.)

For a period of 96 hours following the administration of the test substance there was present in all of the animals soft stools which became formed on the fifth day. Animals autopsied during this study showed no evidence of gross pathology. The material was suspended in olive oil in the proportion of one gm. of sodium carboxymethylcellulose to two ml. of olive oil. Doses delivered to rats were as high as 15 ml. and to guinea pigs as high as 20 ml. Doses higher than this resulted in immediate death owing to mechanical injury and shock from the method of administration.

A comparative study was made on sodium carboxymethylcellulose, locust bean gum, edible gelatin, karaya gum, citrus pectin, and gum tragacanth in order to determine their acute oral-toxicity values. These studies were made on white rats and guinea pigs. The data (Table 2) show that these substances are all within the same range of toxicity when compared from the standpoint of acute oral toxicity. Animals were kept for a two-week observation period following the administration of the test material.

TABLE 2

Acute Oral-Toxicity Study on Gums

Test substance	Number of deaths per 10 animals						
	White rats				Guinea pigs		
	40	30	20	10	15	10	5
	gm./kg.[1]	*gm./kg.*	*gm./kg.*	*gm./kg.*	*gm./kg.*[1]	*gm./kg.*	*gm./kg.*
Gum tragacanth	10	10	2	0	3	0	0
Citrus pectin	8	2	0	0	2	0	0
Karaya gum	10	6	0	0	5	0	0
Edible gelatin	3	0	0	0	2	0	0
Locust bean gum	8	4	0	0	3	0	0
Sodium carboxymethylcellulose	10	5	0	0	3	0	0

[1] It was deemed inadvisable to use higher dosages because of death owing to mechanical injury and shock from methods of administration. Sodium carboxymethylcellulose is considerably more hydrophilic than the other substances tested and therefore the bulk was considerably greater.

CHRONIC ORAL-TOXICITY STUDIES

Six-Month Studies: Two hundred and forty white rats and 240 guinea pigs equally divided as to sex, which had been kept for a two-week observation period to insure normalcy, were allocated as follows: 100 animals of each species received 0.5 gram per kilogram of body weight, 100 of each species received one gram per kilogram of body weight. Forty animals of each species were kept as controls. The test material, mixed with

the regular diet, was given to the experimental animals daily seven days per week for six months. The control animals were handled exactly as were the experimental animals, except that they did not receive the test substance in their food. All of the food supplied was consumed.

Weight Records: Monthly weight records for each animal were kept. These showed normal weight increase except that in some cases experimental animals showed greater weight increases than the control animals.

Fertility Records: Fertility records show that ingestion of the test material for six months did not affect the fertility of the animals.

Urine Examinations: Urine examinations were made on groups of five animals for each species for each dosage group as well as for one group of animals of each species for the control group. These studies involved biochemical tests for sugar, albumin, and phosphates as well as microscopic examination for casts and blood cells. These studies gave no evidence of pathology.

Blood Examination: Complete blood studies were made on each of 10 animals of each species at the beginning of the experiments, at the end of three months, and at the end of six months. These studies determined hemoglobin percentage, leucocyte, and erythrocyte counts as well as differential counts. No evidence of pathology was found.

Histopathology: Five rats and five guinea pigs from each dosage group as well as three rats and three guinea pigs from the control groups were autopsied each month (156 animals during the six-month study). Sections of liver, kidney, spleen, stomach, intestine, and heart were taken and prepared for histopathological study.

There was no gross pathology in the autopsied animals. There was no microscopic pathology present in any of the tissues studied which could be attributed to the sodium carboxymethylcellulose.

Recovery Studies: Five animals of each species in each dosage group were put aside each month for recovery effects in the event damage would be produced. Since there were no pathological changes attributed to the material on monthly examination, it was deemed unnecessary to do histopathological studies on the recovery group since the material may be considered harmless physiologically. It was also deemed unnecessary to do resistance studies on the surviving animals.

One-Year Studies: Twenty guinea pigs were fed 0.5 gram and 20 others were fed one gram per kilogram of carboxymethylcellulose daily in their diet for one year. A control group of 15 guinea pigs was used in the study. Monthly weight records were kept and showed a normal increase in the experimental as well as in the control animals. Twenty animals in both Group I and Group II were autopsied at the end of the first year; there was no gross pathology in any of these animals. Sections of liver, kidney, spleen, heart, stomach, and intestines were prepared from each animal for histological study. There was no pathology in the tissue studied in these animals which could be attributed to the substances tested. Fifteen control guinea pigs were autopsied at the end of one year. Sections of liver, kidney, spleen, heart, stomach, and intestine were prepared from each animal; there was no evidence of gross or histopathology in any of them.

PHYSIOLOGICAL EFFECT OF SODIUM CARBOXYMETHYLCELLULOSE IN DOGS

Three groups of 10 animals each were used in this study. The first group received 0.5 gm. per kg. of sodium carboxymethylcellulose daily in the diet for six months. The second group received one gm. per kg. of sodium carboxymethylcellulose daily in the diet for six months. The third group of dogs received the same diet except for the sodium carboxymethylcellulose and were kept under the same conditions as the experimental animals.

The dogs used in this study were obtained when they were 12 weeks old and were kept for an observation period of one month before starting the experiment. None of the animals in either experimental or control groups died during the course of this investigation.

Weight curves for experimental and control animals were normal. None of the animals exhibited any untoward reactions during the course of the experiment. As a precautionary measure, however, each dog received two distemper injections.

At the end of six months six animals from all three groups were autopsied and the following tissues removed for histological study: stomach, intestines, spleen, liver, kidney, heart, lung, and pancreas. There were no deviations from the normal controls observed in any of these tissues.

From the above information it is apparent that sodium carboxymethylcellulose in the doses given produced no evidence of permanent or chronic injury in dogs.

PHYSIOLOGICAL EFFECT OF SODIUM CARBOXYMETHYLCELLULOSE IN WHITE RATS

Four groups of 25 animals each, approximately equally divided as to sex, were used in this study. All the rats used in this study were obtained immediately after being weaned. Group I received one gram per kilogram of body weight of sodium carboxymethylcellulose daily in their diet for over two years; Group II received 0.5 gram per kilogram of body weight of sodium carboxymethylcellulose daily in their diet for over two years; Group III received 0.1 gram per kilogram of body weight of sodium carboxymethylcellulose daily in their diet for over two years; and Group IV were controls receiving the same diet as the first three groups with the omission of sodium carboxymethylcellulose.

The weight curves of the four groups were normal except that those groups receiving sodium carboxymethylcellulose in the diet showed a slight increase in weight over the control animals.

Monthly urine examinations for albumin, casts, and sugar revealed no deviation from the control group.

Monthly blood studies, such as hemoglobin determinations, red and white cell counts, and differential counts, revealed no deviation from the control group.

Fertility: Litters produced by the experimental as well as the control animals were kept in their respective groups and placed on the same diet as the parents. This was carried out into the third generation and these animals also showed no deviation from the animals in the control group.

At the end of 25 months 12 of the animals in each group were autopsied. There was no gross pathology evident except for an occasional hydatid liver cyst which occurred both in the experimental and control animals.

Microscopic Pathology: Sections of heart, liver, kidney, stomach, intestine, spleen, and adrenal were prepared for histological study. No pathology was found in any of the experimental animals which could be differentiated from that found in the control animals. This pathology was the usual type found in animals which had reached the period of senility. No neoplasms were found in any of the experimental animals. In one control animal a small granuloma was found in the liver.

Nothing was observed during the course of this investigation which continued over the lifetime of the experimental animals and carried on in two generations of offspring, which in any way indicated a deviation from the control animals.

From the above information it is evident that this study further supports the evidence available to date as to the chronic safe use of sodium carboxymethylcellulose by mouth.

RECLAMATION OF SODIUM CARBOXYMETHYLCELLULOSE

Studies to reclaim sodium carboxymethylcellulose from the feces of rats which were fed a known quantity of the material were undertaken. The feces collected were pooled. Five rats were fed five grams of sodium carboxymethylcellulose in one dose, collectively. The feces were collected at 24-hour intervals, and an analytical chemical method was devised for determining the quantity of sodium carboxymethylcellulose which was reclaimed. This method showed that approximately 90 per cent of the sodium carboxymethylcellulose fed to the rats was reclaimed in the feces.

It is our belief that further refinements in the analytical method and in the collection of the feces will lead to a higher percentage of recovery than that reported. It is therefore evident that sodium carboxymethylcellulose is apparently not absorbed from the intestinal tract and is eliminated almost completely.

SKIN IRRITATION AND SENSITIZATION STUDY

The patch-test method employed is a modification of that suggested by Dr. Louis Schwartz [Schwartz *et al.* (1940)] of the United States Public Health Service. Two hundred unselected human subjects, 100 males and 100 females, were used in this study. The test material was placed upon lintine discs[2] covering an area of approximately one and one-half square inches. These patches were placed in direct contact with the skin of the back, covered with waxed paper extending beyond the area of the patch, and held in place by means of adhesive tape. Patches were allowed to remain on the backs of these subjects for a period of five days, at the end of which time the patches were removed and the reactions noted.

Three weeks after the removal of the first patch application the material was again applied to the backs of the same subjects and allowed to

[2] A round cotton disc (1½ inches in diameter) used for milk sedimentation tests, made by Johnson and Johnson.

remain in contact with the skin for 48 hours. At the end of this time the patches were removed and the reactions noted.

There was no evidence of irritation after the first or second application on any of the subjects, indicating that sodium carboxymethylcellulose is not a primary skin irritant and does not appear to be a sensitizing substance.

CLINICAL STUDIES

Sodium carboxymethylcellulose in powder form was used as a vehicle for an antiseptic substance in 134 cases treated for vaginal infections of several types. Five grams of the material was used for each subject. There was no evidence of irritation or untoward reactions to the vaginal mucosa or external genitalia in any of these cases.

SUMMARY

Sodium carboxymethylcellulose, gum tragacanth, citrus pectin, karaya gum, edible gelatin, and locust bean gum are within the same range of acute oral toxicity.

Sodium carboxymethylcellulose was fed to the white rats and guinea pigs in the dosage groups of one-half gram per kilogram and one gram per kilogram of body weight daily in their diet for six months. Animals were autopsied at monthly intervals and sections of liver, kidney, heart, stomach, spleen, and intestine were taken for histological study. There was no gross or microscopic pathology in any of these animals which could be attributed to the substance tested.

Monthly weight records showed normal weight increases in experimental as well as in control animals. Monthly urine determinations on pooled specimens showed normal urine findings. These studies involved tests for sugar, albumin, and phosphates as well as microscopic examination for casts and blood cells. Complete blood studies on representative animals showed no pathological changes. These studies included hemoglobin percentage, leucocyte, and erythrocyte as well as differential counts. Complete fertility records indicated normal fertility rates.

Sodium carboxymethylcellulose was fed to a group of guinea pigs for a period of one year in dosages of one-half gram and one gram per kilogram of body weight. All of the animals were autopsied at the end of a year and sections of liver, kidney, spleen, intestine, stomach, and heart were prepared for histological studies. There was no gross or microscopic pathology in any of the animals which could be attributed to the test substance. Monthly weight records indicated normal weight curves.

Sodium carboxymethylcellulose was fed to a group of dogs for a period of six months in dosages of 0.5 gram and one gram per kilogram of body weight. Six animals from each group were autopsied at the end of six months and sections of stomach, intestine, liver, kidney, heart, lung, and pancreas were removed for histological study. There was no gross or microscopic pathology in any of the animals which could be attributed to any of these substances. Monthly weight records indicated normal weight curves.

Sodium carboxymethylcellulose was fed to a group of white rats for a period of 25 months in dosages of 0.1 gram, 0.5 gram, and one gram per

kilogram of body weight. Twelve of the animals were autopsied at the end of 25 months and sections of heart, liver, kidney, stomach, intestine, spleen, and adrenal were prepared for histological studies. No pathology was found in any of the experimental animals which could be differentiated from that found in any of the control animals. No neoplasms were found in any of the experimental animals. Monthly weight records indicated normal weight curves. Monthly blood studies indicated no pathological changes.

Sodium carboxymethylcellulose when applied to human skin is not a primary irritant nor does it appear to be a sensitizer.

Sodium carboxymethylcellulose when applied to the vaginal mucosa and external genitalia does not cause any irritating effect or any untoward reaction.

CONCLUSION

Sodium carboxymethylcellulose shows no evidence of being toxic to white rats, guinea pigs, dogs, or human beings. It is, therefore, believed that there is no evidence why these materials may not be incorporated into foods or for pharmaceutical preparations designed for human or animal consumption.

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VITAMIN C CONTENT OF HOME-CANNED TOMATOES AS DETERMINED BY VARIETY AND METHOD OF PROCESSING

MARY M. CLAYTON, E. FRANCES PRESSEY, AND KATHERINE H. LEES
Maine Agricultural Experiment Station, Orono, Maine

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Varietal differences in percentage loss of vitamin C in tomatoes during canning and storage were shown by Monroe and Murphy (1943). Their study indicated that two varieties which were initially high in vitamin C (Farthest North C and Mexico) lost a lower percentage of the vitamin during canning and storage for eight months than one variety which was initially low (Farthest North A). Clayton, Goos, Wells, and Murphy (1944) and Clayton, Wells, and Goos (1945) also noted varietal differences in percentage loss of vitamin C in their studies of 18 varieties of tomatoes. Their experiments in 1945 also seemed to show some relationship between the percentage rate of vitamin C loss and the way the tomatoes were grown, whether pruned and staked or grown on the ground.

The work here reported was carried out in 1945-46 in order to obtain additional information on the effect of variety, staking, type of container, method of processing, and storage temperature on the rate of vitamin C loss. Twenty-five varieties were used (Table 1). These varieties were chosen on the basis of their vitamin C content or because they were of particular interest for other qualities, such as earliness, high yield, size, resistance to disease, flavor, and color. All varieties which were thought to be suitable for pruning and staking were grown in this way in order to promote maximum formation of vitamin C by exposure of the fruit to the sun.

Two varieties, John Baer and Stokesdale, were grown both on the ground and on stakes in order to determine how much difference the staking would make in the vitamin C values secured in the raw and canned tomatoes.

EXPERIMENTAL PROCEDURE

Variety Studies: For the studies of varietal differences in vitamin C loss and the effect of type of container and storage temperature, from one to three batches of each variety of tomatoes were canned by the method recommended by the U. S. Bureau of Home Economics.[1] The details of the procedure used were as follows: For one batch approximately one peck (14 lb.) of freshly picked tomatoes were stemmed, washed, and wiped. After being weighed, the tomatoes were scalded for 20 seconds and cold-dipped for 20 seconds (about eight at a time in a wire basket). Cores, skins, and injuries were removed with a sharp, stainless-steel knife and thin, wedge-shaped slivers were cut from two opposite sides of each

[1] Home canning of fruits and vegetables. U. S. Dept. Agr. AWI 93. May, 1944.

TABLE 1

TABLE 1

Varietal Differences in Size and Vitamin C Content of Raw and Canned Tomatoes—1945 Season

	Variety [1]	Av. wt.	Number of batches	Av. raw value (skins removed) vitamin C	Av. canned value Test 1—(1 day) vitamin C		Av. canned value—Test 3 (8 months) vitamin C					
					Tin (r) [2]	Glass (r)	Tin (r)	Lost [3]	Glass (r)	Lost	Glass (c) [2]	Lost
		oz.		*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*
G	Farthest North C-15	1¾	1	32.5	32.0	32.9	30.3	7.1	25.9	20.4	27.9	14.4
S	Golden Queen	4½	3	32.3	30.7	30.7	28.2	12.7	24.2	25.1	25.7	20.3
S	Comet	4	3	31.3	29.9	29.7	28.4	9.6	23.5	24.6	25.1 (2) [4]	22.4 (2) [4]
G	Farthest North C-20	2	3	31.1	29.9 (2) [4]	28.0 (2) [4]	29.6	6.0	22.0	30.2	24.4	21.8
S	Trellis 22 [5]	4¼	3	30.6	31.0	29.7	28.0	8.6	22.7	26.0	25.0	18.5
S	Burpee's 5500	5¾	2	29.1	26.9	25.2	23.8	18.4	18.1	37.7	18.0	38.1
S	Waltham Forcing [6]	3½	2	28.5	28.3	27.1	27.1	5.3	20.5	28.2	21.5	24.8
S	Early Trellis	4¼	3	28.0	29.2	28.4	25.8	8.2	20.3	27.7	21.3	24.6
S	Best of All	4	3	27.1	26.6	25.9	25.3	6.9	20.4	25.0	22.9	15.9
G	Burbank	6	2	26.3	25.6	24.7	24.1	8.8	20.1	23.8	20.3	23.1
S	John Baer	4	3	26.2	26.5	25.3	24.6	6.5	20.4	22.1	22.4 (2)	14.1 (2)
S	Penn. State Earliana	7¼	2	26.0	24.3	23.5	21.8	16.2	19.2	26.3	20.8	39.5
S	Valliant	6	2	25.6	26.2	25.9	24.8	4.0	18.6	28.0	19.3	25.6
S	Stokesdale	5¼	3	25.5	24.6	22.3	23.9	6.6	19.5	23.2	22.0	14.4
G	John Baer	3½	3	24.0	23.0	22.0	21.8	9.5	17.5	27.5	18.9 (2)	20.6 (2)
S	Mingold	4	1	24.0	23.5	23.2	21.9	9.0	16.4	31.5	16.8	29.9
G	Me. 85-87	2	1	23.5	21.0	20.7	20.1	14.5	14.7	37.5	12.2	48.1
S	Master Marglobe	6½	2	23.1	21.2	20.6	20.0	13.9	14.3	38.2	16.7 (1)	28.6 (1)
G	Stokesdale [6]	5	3	23.1	22.2	21.6	21.3	8.1	18.7	19.1	16.9	24.6
G	Me. 85-64	2¾	1	22.1	23.3	21.5	19.9	11.0	14.6	34.8	.....	.....
G	Robson's Victor	6	1	22.1	22.2	19.8	21.0	5.3	16.5	25.4	16.1	27.3
S	Danmark	2½	1	21.5	20.0	20.9	18.2	15.3	16.4	23.4	16.4	23.5
G	Victory	4½	1	21.4	22.3	20.8	18.7	13.0	14.3	33.2	14.8	30.7
S	Danmark x Pritchard	.....	1	20.5	20.4	19.1	18.0	12.9	13.1	36.3	10.5	49.3
S	Fordhook Hybrid	7¼	1	20.4	19.6	19.2	17.2	15.8	11.4	44.4	12.3	40.1
S	Montreal Market	6	1	19.7	16.6	17.5	17.5	11.5	11.7	40.5	12.7	35.6
G	Bounty	6	1	19.6 [7]	19.2	18.3	18.0	8.8	11.4	42.0	11.8	40.2

[1] G=grown on the ground; S=pruned and staked. [2] (r)=room temperature; (c)=cellar temperature. [3] From raw equivalent value. [4] Numbers in parentheses indicate number of batches tested. [5] Last year plants were shaded by a row of corn, resulting in lower vitamin C values. [6] Plants much farther apart than last year. Fruit exposed to more sun, resulting in higher vitamin C values. [7] Average raw value of 22.8 mg./100 gm. secured later in the summer.

tomato. The slivers were dropped immediately into two Waring blendors, each containing approximately 200 gm. of tomato and 200 ml. (202.5 gm.) of five per cent metaphosphoric acid. A weighed aliquot (approximately 35 gm.) of the blended mixture was further extracted with 100 ml. of three per cent metaphosphoric acid and the filtered extract used for the raw analyses. The remainder of each tomato was cut in pieces and dropped into a 14-quart aluminum kettle. The tomatoes were again weighed and then heated, with stirring, until they came to a rolling boil. After being reweighed they were used to fill three No. 2 plain tin cans and seven Mason-type glass jars. The tin cans were filled to within one-fourth inch of the top and the glass jars to within one-half inch. The containers were clean and hot. One-half teaspoon of c.p. sodium chloride was added to each can and jar before filling. The weight of the contents of each was recorded and both the cans and jars completely sealed. The jars were sealed with Crown metal caps which are lined with enamel.[2] These caps allow for partial exhaustion of the air in the jars during processing. Both the glass jars and the tin cans were processed 10 minutes in a boiling-water bath. The tin cans were stored at room temperature and the glass jars at both room and cellar temperatures. Most of the time during storage the temperature of the storage cellar varied between 0 and 10°C.(32 and 50°F.).

Vitamin C analyses were made the day after canning and again four months and eight months later. To prepare the composite sample of canned tomatoes 200 gm. of the mixed contents of each can or jar and 200 ml. (202.5 gm.) of three per cent metaphosphoric acid were blended in a Waring blendor. An aliquot of this mixture weighing approximately 35 gm. was further extracted with 100 ml. of three per cent metaphosphoric acid and the filtered extract used for the analysis.

The basic xylene method of Robinson and Stotz (1945) was used for the analyses of both the raw and canned tomatoes.

Special Comet Series: For the studies of the effect of eight variations in canning procedure only the Comet variety was used; these variations are outlined (Table 2). In this series the tomatoes were prepared and precooked as previously described. The variations used were only in the type of container, type of lid, type of seal, method of processing, etc. Five batches of tomatoes (12 lb. in each) were canned and the eight variations in procedure used in each batch. Vitamin C analyses were made after storage for six months at room temperature.

RESULTS OF VARIETY STUDIES

Raw Values for Vitamin C: The raw values ranged from 32.5 mg. (per 100 gm.) for the Farthest North C-15 variety to 19.6 mg. for the Bounty variety (Table 1). Of the 10 varieties highest in vitamin C all except the two strains of Farthest North C were grown on stakes. The Burbank variety tested the highest in vitamin C of any tomato of good size grown on the ground. The average weight of the Burbank tomatoes was six ounces, while the Farthest North C-20 variety averaged only two ounces. The even smaller size of the Farthest North C-15 variety would

[2] The sealing compound used on the Crown caps was not found entirely satisfactory.

be considered objectionable; however, the flavor and quality of both strains are excellent. Of the high vitamin C varieties grown on stakes the quality and flavor of the Comet tomato were best. The plants of this variety bore huge clusters of bright red fruit, each averaging four ounces in weight.

Raw Values—Effect of Staking Versus not Staking: The raw values obtained for the John Baer and Stokesdale varieties (Table 1) showed that staking raised the vitamin C content of the tomatoes about two mg. per 100 gm. This difference owing to staking, although small, was statistically significant. The plants grown on the ground were spaced four feet apart, which allowed fairly good exposure to the sun. This wide spacing was apparently responsible for the fact that vitamin C values for the tomatoes grown on the ground in 1945-46 were considerably higher than those secured the previous summer when the plants were closer together.

TABLE 2

Effect of Variations in Canning Procedure on Vitamin C Content of Canned Tomatoes (Comet Variety)

Procedure	Vitamin C[1]
	mg./100 gm.
1. Atlas strong-shoulder Mason glass pint jar with glass lid and metal screw band. Complete seal during processing for 10 minutes in water bath	18.84
2. Same as Procedure 1 only with partial seal	26.09
3. Atlas strong-shoulder Mason glass pint jar with Crown metal cap and metal screw band. Ten-minute processing time in water bath	24.34
4. Same as Procedure 3 except 20 minutes processing time	24.63
5. Same as Procedure 3 except processed 10 minutes in a pressure canner at 10 pounds pressure	26.15
6. Same as Procedure 3 with the addition of 150 mg. of vitamin C in tablet form	53.50
7. Same as Procedure 3 except with a 1½-inch square of tin (from a plain tin can) added	27.01
8. No. 2 plain tin can. Processing time 10 minutes in water bath	28.88

[1] Tested six months after canning; averages for five batches.

Canned Values—Test 1—One Day After Canning: On the day after canning the average vitamin C values secured for the tomatoes canned in tin were practically the same as the raw values. For those canned in glass the average values, in almost all cases, were very slightly lower than for those canned in tin (Table 1).

Canned Values—Test 2—Four Months After Canning: The tests made on the canned tomatoes stored at room temperature showed that those in tin cans had lost practically none of their vitamin C. Those in glass jars had lost from 11.8 to 37.5 per cent of the vitamin C originally present in the raw tomatoes. In most cases the losses varied according to the original vitamin C content of the raw tomatoes, the highest vitamin C varieties showing the lowest percentage losses.

Canned Values—Test 3—Eight Months After Canning: Results of the tests made at the end of eight months showed that in the tin cans and glass jars stored at room temperature the vitamin C losses were significantly higher in the glass jars. The highest vitamin C varieties again showed the lowest percentage losses (Table 1).

A comparison of the average percentage losses at the end of four months and eight months for the tomatoes canned in glass showed that the greatest losses occurred during the first four months.

Canned Values—Effect of Storage Temperature: The vitamin C values for the tomatoes (canned in glass) stored for eight months in a cold cellar storeroom were significantly higher than for those stored at room temperature (Table 1).

Canned Values—Effect of Staking Versus Not Staking: The canned values for the staked John Baer and Stokesdale tomatoes were significantly higher than those for the same varieties grown on the ground (Table 1).

RESULTS OF SPECIAL COMET SERIES

Effect of Variations in Canning Procedure: A statistical comparison of the results obtained in this series showed highly significant differences between the following pairs of procedures (Table 2):

1 and 2: Glass pint Mason jars with glass lids and metal screw bands. Processed 10 minutes in a boiling-water bath. A partial seal during processing gave significantly higher vitamin C values than a complete seal.

3 and 7: Glass pint Mason jars with Crown metal caps and metal screw bands. Processed 10 minutes in a boiling-water bath. The addition of a one and one-half-inch square of tin to the jars resulted in significantly higher vitamin C values.

2, or 3, and 8: Glass pint Mason jars with glass lids and metal screw bands (partial seal), or the same jars with Crown metal caps, versus No. 2 plain tin cans. The tomatoes in tin were significantly higher in vitamin C than those in glass.

5 and 8: Glass pint Mason jars with Crown metal caps (processed 10 minutes at 10 pounds pressure) versus No. 2 plain tin cans (processed 10 minutes in a boiling-water bath). The tomatoes in tin were significantly higher in vitamin C.

7 and 8: Tomatoes canned in tin cans were significantly higher in vitamin C than those in glass jars (with Crown caps) containing a piece of tin.

The high values obtained for Procedure 6 indicate that the vitamin C added in the form of tablets was well retained. This suggests a simple way to make tomato juice the equivalent of orange juice in vitamin C value or to increase the vitamin C content of products made from low vitamin C varieties of tomatoes.

DISCUSSION

Since home-canned tomatoes and tomato juice are often used as principal sources of vitamin C in the diet it seems worth while to list the principal factors which are known to affect the vitamin C content of these products.

First may be mentioned the variety factor, as it affects the vitamin C content of the raw tomatoes. Murphy (1942) pointed out that, under a given set of growing conditions, individual varieties of tomatoes usually maintain their relative positions with respect to vitamin C content. Therefore, in order to secure home-canned tomatoes which contain significant

amounts of vitamin C, even after storage for as long as eight months, it seems important to use varieties such as Comet, which are initially high in vitamin C. As an alternative, vitamin C tablets can be added to jars of low-vitamin tomatoes at the time of canning.

A second important factor for high vitamin C tomatoes is exposure of the fruit to the sun during ripening. The effect of sunlight in increasing the vitamin C in tomatoes was first shown by Veselkine *et al.* (1934). This effect has since been noted by many other workers. One of the most important recent studies is the one by Hamner, Bernstein, and Maynard (1945). These authors state that by far the greatest influence on the vitamin C content of tomatoes of a given variety is produced by variations in light intensity previous to harvest. In a home garden exposure to the maximum amount of sunlight may be obtained either by pruning and staking (or otherwise supporting the plants) or by the wide spacing of plants grown on the ground. Commercial growers would find only the latter method practical.

In 1944-45 the writer found that the high vitamin Farthest North C tomatoes grown on the ground lost as high a percentage of their vitamin C during canning and storage as several of the low vitamin C varieties which were grown in the same way. Since the percentage losses in the Farthest North C variety were much lower this year, the results for last year can be explained only as being due to the poor quality of the tomatoes. They were quite badly cracked as the result of previous dry weather.

The third important factor is the method of processing. In the home canning of tomatoes consideration should be given to the following:

1. Adequate precooking in order to remove air and destroy enzymes.

2. Working entrapped air out of the containers before processing. This can be done with a silver knife.

3. Use of a minimum amount of head space in jars or cans.

The importance of minimum head space was pointed out by Tressler and Curran (1938) and Esselen and Woodward. Although the "lightning type" of home-canning jar is popular with housewives, a container with a flat top makes it possible to use a smaller head space.

4. Use of tin containers (or possibly glass jars with tin-lined caps or tin insets).

The experiments reported in the present paper show that the highest retention of vitamin C was obtained in tin cans. This has also been shown by Daniel and Rutherford (1936), Hauck (1938), and others. Hussemann (1935) reported increased retention of vitamin C in bottles with metal caps. Reynolds (1938) successfully used tin insets. Tin-lined bottle caps have been used to some extent by commercial canners but tin-lined jar caps for use in home canning are not yet available on the market. In regard to the use of tin insets, it is a simple matter to cut them from new plain tin cans. However, it is probable that the average housewife would not take the time to cut and use them.

The effect of the tin in preventing vitamin C oxidation can probably be explained as being due to the reducing action of traces of tin salts.

5. Use of a processing time long enough to exhaust most of the air from the containers.

In the present study somewhat better average retention of vitamin C was obtained by processing the tomatoes for 10 minutes at 10 pounds pressure, rather than for 10 minutes in a boiling-water bath. However, statistical analysis of the results showed no significant differences owing either to increased processing time in the water bath or to the use of a pressure canner (Table 2).

A fourth factor which is of some importance in the retention of vitamin C in home-canned tomato products is storage temperature. However, unless the temperature of the storage cellar averages below 15.6°C.(60°F.) the containers may as well be stored at room temperature, Clayton, Wells, and Goos (1945).

SUMMARY

Percentage loss of vitamin C during canning and storage was studied in 25 varieties of tomatoes which ranged in raw value from 32.5 to 19.6 mg. per 100 gm. The results of the tests on the canned tomatoes made at the end of eight months showed that the vitamin C values were significantly higher in No. 2 plain tin cans than in pint-size glass jars. The highest vitamin C varieties showed the lowest percentage losses. The values for the tomatoes (canned in glass) stored for eight months in a cold cellar storeroom were significantly higher than for those stored at room temperature.

The effect of staking versus not staking on raw and canned vitamin C values was studied in the John Baer and Stokesdale varieties. The results showed that staking produced significantly higher vitamin C values in both raw and canned tomatoes.

Experiments on the effect of variations in canning procedure showed no significant increase in canned vitamin C values as the result of increasing processing time in the water bath to 20 minutes or as the result of processing for 10 minutes at 10 pounds pressure. Vitamin C values for tomatoes canned in plain tin cans, or in glass pint jars with tin insets, were significantly higher than for those in pint jars without the tin insets. Vitamin C in tablet form, added to the tomatoes before processing, was well retained after storage for six months.

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THERMAL DEGRADATION OF DEHYDRATED BEETS[1]
I. ANALYTICAL PROCEDURE

E. M. ARONOFF AND S. ARONOFF[2]
Department of Chemistry, University of Chicago, Chicago, Illinois

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The purpose of the following investigation was to determine the nature of the thermal degradation of dehydrated beets. It was felt necessary, for this purpose, to make a somewhat extensive analysis of the primary constituents of beets degraded at various temperatures and to note the changes, if any, in these constituents.

It is common knowledge that the beet root is not only an effective storage organ but is also an active metabolizer of nitrogenous compounds. Analytical procedure was therefore divided, roughly, into carbohydrate and nitrogenous constituents and included two different varieties of beets, Egyptian Crosby and Detroit Red. The total percentage of relatively well-known organic substances which were routinely determined was 80 to 90 per cent.

Temperature conditions—control 25°C.(77°F.), samples 40, 50, 60, and 70°C.(104, 122, 140, and 158°F.)—were predicated on the facts that even the highest temperature is not impossible under certain desert conditions, and samples of beets stored in cans under normal conditions for lengthy periods (two to three years) showed strong resemblance to highly degraded material.

It was shown at an early stage that the nature and rate of the deterioration is a function of the water content. The degradative process was permitted to occur under saturating water vapor at the temperature indicated, the water contents, after 16-day incubation, ranging from 4.6 per cent (40°C.) to 11.5 per cent (70°C.). Data are also included for material in a sealed vessel treated for various periods (two, four, and six weeks) without addition of aqueous vapor at 50°C.; in addition, a sample is included which was degraded in the open for a 16-day period at 60°C. In the aquothermal degradation the external appearance of the beets changes from the normal dark red through orange-brown to brown and finally black. The color of the material heated in the open does not proceed beyond a light brown, while that in the sealed vessels shows a change, though not so drastic, similar to the aquothermal.

EXPERIMENTAL METHODS

Symbols: The following abbreviations are used: DR=Detroit Red variety of table beets. EC=Egyptian Crosby variety of red beets. Nu-

[1] The subject matter of this paper was undertaken in co-operation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

[2] Present address: University of California, Radiation Laboratory, Berkeley 4, California.

merals 40, 50, 60, and 70 alone indicate the temperature of degradation in saturated water vapor; time is 16 days. 60op = degradation at 60°C. in the open for 16 days. The number 50 followed by —2wk, —4wk, or —6wk indicates the length of time (two weeks, four weeks, or six weeks, respectively) of degradation of beets sealed in tubes without addition of water.

Thus: DR-50 = Detroit Red beet incubated in saturating water vapor for 16 days. EC-50-4wk = Egyptian Crosby beet incubated for four weeks at 50°C. in sealed tube.

All samples were ground in a Wiley mill to facilitate more homogeneous sampling.

Water: Water was determined as the weight loss resulting from heating the samples in a vacuum oven at 60°C. for 18 hours at a pressure of approximately 0.1 mm. Hg. Additional water was not lost by increasing the time of dehydration. The use of an alternative method, e.g., titrimetrically with K. Fischer reagent, was precluded by the intensity of color.

Ash: The per cent of ash was obtained by heating the material in a muffle furnace, the temperature of which rose from 200 to 600°C.(392 to 1112°F.) in about four hours, following which the temperature was kept constant for an additional two hours.

Spectroscopic data (Table 2) showed that even at this relatively low temperature volatile constituents, such as zinc, are lost. Indeed the entire method of dry ashing is still open to the question of rate of loss of the various elements. The semiquantitative determination of the nonvolatile matter was performed spectrographically in the usual manner.

Sugars: Sugars were determined by the method of Hassid (1936) with a few slight modifications. The samples were extracted for 14 hours in 80 per cent ethanol in a modified Soxhlet. They were clarified with lead acetate (excess lead was precipitated with potassium phosphate) and charcoal (Nuchar CA 39). If the charcoal is omitted, sufficient Pb ion is present to add a small but significant error to the titer; furthermore, in the case of the beet considerable yellow color remains. Not all the color can be removed even by this means in strongly degraded material.

When filtering, the charcoal was carefully washed with hot water (60-75°C.) to avoid loss of glucose which showed some tendency to be adsorbed on the charcoal.

The amount of sugar was then determined by potassium ferricyanide reduction which was titrated with ceric sulfate. Setopaline C was used as the indicator.

Starch: Starch was run on the sugar-extracted residue. Samples were incubated for 24 hours at 37°C.(98.6°F.) with taka-diastase, as suggested by Morrow (1927). The reducing sugar formed was then determined by ceric sulfate titration, Hassid (1936), and the per cent of starch calculated from the simultaneously determined value of a known sample of starch.

Lignin: The determination of lignin was performed at two different stages with somewhat different results. One experiment used the residues obtained from the determination of pectic substances; the other, that from the starch determinations. The method used was that recommended by

Manning and DeLong (1941) in which the sample was allowed to hydrolyze in 72 per cent sulfuric acid at 10°C.(50°F.) for two hours, following which the solution was diluted to three per cent sulfuric acid, stirred thoroughly, heated almost to boiling for three hours, and filtered. The following is a comparison of the results obtained (expressed as per cent of dry weight):

Results	Pretreatment	
	Extracted 14 hr. in 80% alcohol; boiled 45 min. in weak citric acid, pH 3-4	Extracted 14 hr. in 95% alcohol; boiled 45 min. in weak acetic acid, pH 4
	pct.	*pct.*
DR	2.72	2.72
DR-40	1.94	1.35
DR-50	2.44	0.78
DR-60	4.92	3.00
DR-70	.....	13.15
EC	2.69	2.69
EC-40	3.01	1.12
EC-50	2.96	1.00
EC-60	4.95	3.14
EC-70	9.05	8.88

Since the method employed is a residual one, that is, the product is not extracted and determined specifically but it is assumed that all else is removed, one may presume that the method giving lowest values is most satisfactory.

Pectic Substances: The sample was extracted in a modified Soxhlet for 14 hours with 95 per cent alcohol, the residue being then boiled with citric acid solution (13.15 gm. per liter, or approx. 0.06 M) for 45 minutes, and filtered hot. The pectic substances were then precipitated (overnight) with four times the volume of acidified ethanol (1.24 N HCl in 95 per cent alcohol), filtered, washed with water, dried, and weighed back. It is to be noted that values for the pectic substances are rather variable. The higher values were often associated with a pronounced discoloration which could not be removed by repeated solvation and reprecipitation. This indicates the presence of foreign matter either adsorbed upon or conjugated with pectic substances.

The method is essentially that of Säverborn (1945), with slight modifications, which, although capable of yielding practically pure pectin with some materials, does not go beyond the claim of preparation of the class of pectic substances in general.

Cellulose: The method employed was essentially that of Wise, Peterson, and Harlow (1939). The residue from the determination of pectic substances was refluxed for five hours in ethanolamine. After dilution with water the cellulose was filtered in a sintered glass filter, washed, covered with Clorox for five minutes, washed thoroughly with a weak acid, washed with water, dried, and weighed back. The use of additional oxidizing and decolorizing agents was found to be unnecessary in nonwoody tissue.

Organic Acids: Organic acids were determined by method of Pucher, Vickery, and Wakeman (1934). The following are values obtained:

Variety	Oxalic acid	Malic acid	Citric acid
Egyptian Crosby	Trace	1.46	0.13
Degraded EC	Trace	0.66	0.19
Detroit Red	Trace	1.20	0.86

Total (Kjeldahl) Nitrogen: The material was digested until clear, using the catalysts suggested by Clark (1943), i.e., concentrated sulfuric acid containing potassium sulfate and mercuric oxide, following which one drop of ethanol was added and the samples heated one-half hour after clarity. Repeated experiments with other types of catalysts, e.g., selenium and its oxides, gave results which were low with red beets.

Following the digestion the sample was then distilled into three per cent boric acid after being made alkaline in a Kirk-type (1936), all-glass, micro-Kjeldahl distillation apparatus and titrated with a double indicator (five parts of bromcresol green to one part of methyl red) as suggested by Ma and Zuazaga (1942).

Ammonia and Amide Nitrogen: Pucher, Vickery, and Leavenworth (1935) outlined a method for ammonia and amide nitrogen that was followed almost exactly. The sample was first distilled in a buffered solution of phosphate under vacuum. The remaining solution was filtered, hydrolyzed, and redistilled to give amide nitrogen. The color developed by Nessler's reagent was read on a Klett colorimeter, using Corning filters No. 3389 and 5030 (the choice of which was predetermined by examination of the absorption spectrum of the orange color formed in the reaction).

Amino Nitrogen (ninhydrin reaction): The method of Van Slyke, Dillon, MacFayden, and Hamilton (1941) was modified somewhat. Carbon dioxide was evolved from the sample by the addition of ninhydrin in the presence of citrate buffer, as prescribed by the above authors. For this purpose the sample and reagents were placed in a test tube connected in series with another test tube containing 10 ml. of barium hydroxide of a concentration suitable to absorb all of the carbon dioxide and still give a titer of over five ml. The apparatus was patterned after that suggested by Folin and Farmer (1912) for use with either suction or air pressure.

The sample was boiled for exactly 15 minutes as CO_2-free air was slowly bubbled through to sweep the carbon dioxide into the alkali. After the addition of acetone, as suggested by Christensen, West, and Dimick (1941), it was titrated with hydrochloric acid of approximately half the normality of the barium hydroxide, using thymol blue as the indicator.

Because of the specificity of the reaction only the amino-acid nitrogen is determined by this method, and considerable nitrogen, much of which is probably amino, remains undetermined.

Protein Nitrogen: The protein nitrogen was obtained by determination of the additional amino-acid nitrogen in the sample after hydrolysis

in 6 N sulfuric acid for a period of 14 to 24 hours. Because of the difficulty of determining the exact time necessary for maximum hydrolysis and minimum formation of "humin," owing to the large amount of glucose present, a group of samples was hydrolyzed for different lengths of time. The deteriorated samples showed an appreciable change in the time of hydrolysis necessary to obtain maximum protein nitrogen (see Discussion). Several samples were run to determine the optimum period of hydrolysis for each; the time was found to decrease from 24 hours in untreated samples to 14 hours in samples treated at 70°C.

DATA AND DISCUSSION

For convenience the discussion is divided into three sections: general and inorganic, carbohydrate fraction, and nitrogenous fraction.

General and Inorganic Composition: The over-all composition of the two varieties of red beet used is given (Table 1), the results being ex-

TABLE 1

General Composition of Detroit Red and Egyptian Crosby Beets

Constituent	Detroit Red	Egyptian Crosby
	pct.	*pct.*
Reducing sugars	2.07	5.15
Non-reducing sugars	49.13	47.55
Lignin	2.72	2.69
Cellulose	7.34	7.26
Starch	0.24	0.30
Pectic substances	4.73	4.55
Organic acids	2.16	1.65
Total nitrogen × 6	13.26	12.06
Ash	9.27	10.13
Total	90.92	91.24

pressed as percentages of the dry weight. The two varieties used were grown on adjoining plots and therefore probably on similar soil; they were planted and harvested at the same times.[3] A number of facts are to be noted. The general composition of the two varieties is similar, the outstanding differences being the higher reducing-sugar values of the Egyptian Crosby. The total amount of substance analyzed accounts for 90 per cent or more of the dry weight; about 80 per cent is organic. Simple sugars account for over half the organic matter, and by far the largest fraction of these are non-reducing sugars (primarily sucrose and some raffinose). Nitrogenous compounds comprise almost half of the remainder of the organic fraction and (as will be seen later) proteins approximate 40 per cent of this.

The following data are included: spectroscopic data, dry-ash analyses, water determinations, and gas exchanges. The results indicate that (a) considerable gas exchange occurred, both CO_2 being lost and O_2 consumed, the latter generally to a greater extent than the former; (b) water was

[3] Courtesy of Professor Green, Department of Agronomy, Purdue University.

both "bound" and lost by degradative dehydration; and (c) care must be used in interpretation of ashing data, especially with regard to volatile substances.

A semiquantitative analysis of the inorganic constituents is presented (Table 2), the qualitative aspects of which have given rise to comments concerning missing components earlier in the paper. Of interest is the absence of cobalt in the Detroit Red, of lead in the Egyptian Crosby, and the high concentrations of nickel and silicon in both species. The high concentration of phosphorus is of import in relation to our having qualitatively ascertained considerable phosphatase and pyrophosphate activity in the material.

TABLE 2

Analysis of Inorganic Composition of Detroit Red and Egyptian Crosby Beets

Strength of constituent		Detroit Red	Egyptian Crosby
>1%	Very strong	Ca, Mg, Na, (K)[1], (S)[1]	Ca, Mg, Na, (K)[1], (S)[1]
	Strong	Al, Si, Fe	Al, Si
1 to 0.01%	Moderate	Ni, P	Fe, Ni, P
	Weak	Ba, Cu, Li, Ti	Co, Cu, Li, Mn
<0.01%	Very weak	B, Mn	B, Ba, Ti
	Trace	Cr, Pb	Cr
	Faint trace		Ag
	Not detected	Ag, As, Be, Ca, Hg, Sb, Sn, Zn,[1] Zr	As, Be, Hg, Pb, Sb, Sn, Zn,[1] Zr

[1] Potassium and sulfur were not tested for but would no doubt appear in the very strong group as indicated. Zinc must have existed as a volatile form which was lost in the ashing process.

The values for ash are given (Table 3), expressed as per cent of dry weight. The results were calculated after due correction for the differing water contents and are in agreement with the trend given by the limiting value of the sample heated open at 60°C.

Variations in the ash content are explained as the result of at least two reactions: (a) the "binding" of water, e.g., that used in hydrolyses, and (b) the material loss in weight of organic substances by CO_2 evolution and pyrolysis (dehydration). The first consideration can be of import inasmuch as an appreciable fraction of the material is subject to hydrolysis, e.g., sucrose, raffinose, and proteins. This process results in a gain in the dry weight and the consequent decrease in the ash contents as reflected in the DR-50, EC-40, and EC-50 samples.

The loss of organic matter is confirmed by an increase above the control samples of other fractions, as total N, cellulose, etc. A diminution of organic matter obviously results in an ash content exceeding the original. The extent of this loss is obtained by subtraction of the ash from the (total) solids content, giving the per cent of organic matter. The result indicates that almost 10 per cent of the organic matter is lost in the most strongly degraded samples of both varieties.

Samples which had been incubated in the open so that the water vapor could escape into the air would, of course, lose all their "unbound" water

in due course of time. The lack of further water loss in the dry-weight determination indicated that this period of incubation was sufficient. Further, the nominal ash content of the "60-open" samples indicates that material organic matter destruction has not occurred; the lack of water, however, makes the percentage of organic matter of these higher than any of the others. In the others the trend indicates the expected increased absorption of water with increasing vapor pressure owing to higher temperatures. It is not known whether equilibrium of the beets and water vapor occurred in the time of incubation; excessive water was, however, present in all cases, so that, for example, even in the 70°C. samples some water remained in the side tube containing the water at the end of the 16-day period.

TABLE 3

Solids, Organic, and Ash Content of the Two Varieties of Beets Used

Variety and treatment	Solids [1]	Organic matter [2]	Ash [3]
	pct.	*pct.*	*pct.*
DR	96.02	88.03	8.32
DR-40	95.38	87.39	8.38
DR-50	94.59	87.12	7.89
DR-60	93.60	85.57	8.58
DR-70	88.40	80.24	9.25
DR-60op	100.00	91.37	8.63
DR-50-2wk	95.80	87.46	8.71
DR-50-4wk	95.60	87.13	8.86
DR-50-6wk	95.48	87.17	8.72
EC	96.06	87.07	9.35
EC-40	95.35	86.81	8.95
EC-50	92.95	84.32	9.29
EC-60	90.43	81.95	9.39
EC-70	88.88	80.33	9.62
EC-60op	100.00	90.68	9.32
EC-50-2wk	95.87	86.96	9.31
EC-50-4wk	95.92	87.13	9.17
EC-50-6wk	95.98	87.05	9.30

[1] Solids=solid content after treating in vacuum oven at 70°C. for 16 hours. [2] Organic matter=per cent solids—per cent ash (unadjusted). [3] Ash=per cent ash after heating at 600°C. for two hours, dry-weight basis.

Material from which oxygen had been removed by 24-hour evacuation on a high-vacuum line still darkened. The darkening is thus not necessarily associated with oxidation. Furthermore, a pressure, presumably due to CO_2 development, was obtained as a function of the temperature, Stadtman, Barker, Haas, Mrak, and Mackinney (1946). Nevertheless, degradations at 70°C. performed in an atmosphere of air developed a slight vacuum, indicating that absorption of oxygen was slightly greater than carbon dioxide evolution, though not sufficiently great to overcome the over-all loss in weight because of the greater molecular weight of carbon dioxide (and pyrolysis of water).

Carbohydrates and Related Substances: Preliminary tests with commercial samples which, as mentioned earlier, deteriorated much more rapidly, indicated an increase in pH of degraded material (e.g., from ca. 5.5 to ca. 4.7). An examination was therefore made of organic acids known (Bennett-Clark) to occur in prominent amounts in red beets, namely malic and citric acids, with a trace of oxalic. As will be seen from the values given under the method for organic acids, a decrease rather than increase in organic acid content of these substances occurs. It is not possible to say whether the increase in pH is due to increase of unknown organic acids or decrease in the effective basicity of the nitrogen in various compounds by masking, e.g., the formation of completely alkylated amines, or by volatilization of amines.

TABLE 4

Sugar Content of the Two Varieties of Beets Used[1]

Variety and treatment	Reducing sugar	Non-reducing sugar[2]	Total sugar	Lignin
	pct.	*pct.*	*pct.*	*pct.*
DR[3]	2.07	49.13	51.2	2.79
DR-40	3.24	41.46	44.7	1.29
DR-50	2.28	44.42	46.7	0.76
DR-60	6.09	38.91	45.0	2.96
DR-70	15.35	16.75	32.1	14.06
DR-60op	3.01	41.99	45.0	2.09
DR-50-2wk	2.14	47.66	49.8	1.05
DR-50-4wk	2.61	45.89	48.5	1.55
DR-50-6wk	2.41	47.59	50.0	2.47
EC	5.15	47.55	52.6	2.67
EC-40	3.67	42.33	46.0	1.09
EC-50	4.48	43.52	48.0	1.01
EC-60	10.47	37.62	48.1	3.14
EC-70	16.00	18.60	34.6	9.07
EC-60op	5.72	43.28	49.0	1.84
EC-50-2wk	3.29	44.91	48.2	0.70
EC-50-4wk	3.47	44.93	48.4	0.84
EC-50-6wk	3.79	45.81	49.6	2.54

[1] All values are for per cent of the dry weight. [2] The values given above for non-reducing sugars include only 50 per cent of all raffinose. Since the raffinose content was shown to be about one to two per cent, the error involved is very slight. [3] The symbols are more fully explained both in Table 3 and the text.

In presenting the data on the most prominent components of dehydrated red beets, the sugars, (Table 4 and Fig. 1), differentiation was not made in the non-reducing sugar fraction between the raffinose and sucrose, the former being known to occur in significant amounts, i.e., one to two per cent.

The following important results are notable: with increasing degradation there is (a) an increase in the reducing sugar, (b) a decrease in the non-reducing sugar, (c) an increase in the apparent lignin content, and (d) a decrease in the total sugar content.

It had previously been shown with the more readily degraded commercial material that under maximal temperature the hydrolysis of sucrose was complete within 24 hours. The process is obviously slower here but would undoubtedly go to completion in sufficiently long treatment. Although invertase is noted as one of the enzymes which may operate under elevated temperature conditions, the conditions both of dehydration and of degradation preclude the nature of the hydrolysis as being due to invertase. It must be nonenzymatic in nature, the catalyst being of unknown nature; possibly the microscopic capillaries formed within the tissue during dehydration provide sufficient surface so that diverse types of catalyses may occur.

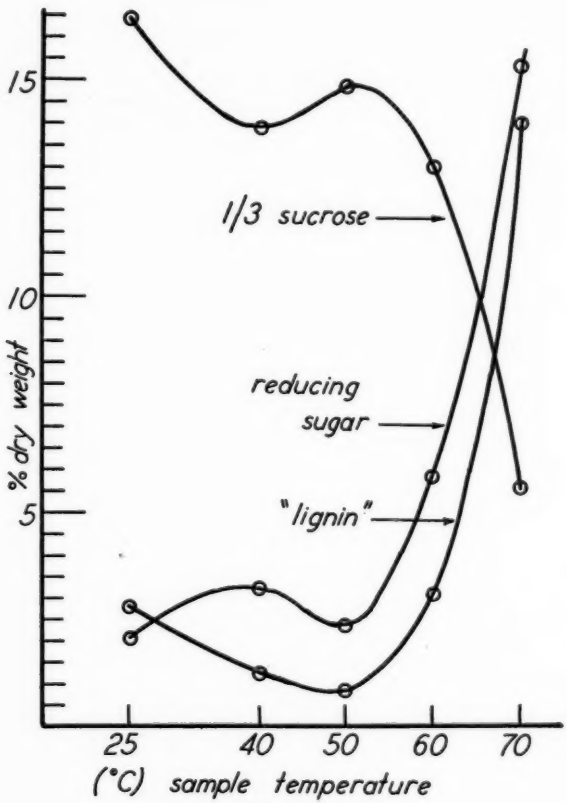

FIG. 1.

That the reducing sugar was almost entirely fructose was shown in the completely degraded (commercial) sample in which sucrose was absent by virtue of the rapidity and intensity of the skatole test and the levorotation in polarized light. In this sample, whose reducing value had previously been determined in a separate portion, the fructose was 87.5 per cent of the reducing sugar. Similarly, in the strongly degraded DR and EC sam-

ples, despite the presence of residual amounts of both glucose and sucrose, the rotation in plane polarized light was levorotatory. Glucose had thus disappeared from the milieu either by conversion to substances such as levulinic acid, or furfural derivatives, or it had combined with other substances also known to be unaccounted for, as $-NH_2$, in some manner as to have lost its reducing power.

TABLE 5

Pectic Substances and Cellulose Content of the Two Varieties of Beets Used[1]

Variety and treatment	Pectic substances	Cellulose
	pct.	*pct.*
DR	4.69	7.28
DR-40	3.66	6.65
DR-50	4.13	6.71
DR-60	3.75	6.75
DR-70	4.33	7.05
DR-60op	2.65	7.41
DR-50-2wk	1.89	7.48
DR-50-4wk	1.86	7.75
DR-50-6wk	1.82	7.83
EC	4.53	7.24
EC-40	2.50	6.96
EC-50	2.18	7.07
EC-60	4.11	7.40
EC-70	4.23	7.78
EC-60op	3.73	7.95
EC-50-2wk	2.27	8.05
EC-50-4wk	1.53	.....
EC-50-6wk	1.77	.....

[1] All values are for per cent of the dry weight.

It is possible, indeed probable, that at least a portion of the material accounted for may be found in the "lignin" fraction. As noted in the methodology, the determination of lignin is extremely unspecific; essentially it is a residual method, the significance of which is limited to a comparison of relative values based upon a uniform technique.[4] Nevertheless such techniques are capable of being very uniform, and the primary question of doubt is the extent of incorporation of nitrogenous residues within the lignin fraction. In this instance, for example, the increase in lignin is accompanied by visible changes in its characteristics—from a light tan, fluffy to a coarse, black material. The material thus produced tends, in all respects, to approach the indefinable "humin." Gortner and Blish

[4] It is hardly necessary to point out that the obvious escape from the dilemma, and one for which many agricultural chemists have undoubtedly sought, is the identification of some unit of the lignin complex, e.g., after appropriate treatment, a vanillin. Comparative data between different plants would, of course, involve the concept of the identity of the lignin molecule as such, a concept which is still in an unsettled state.

(1915) have shown that amino acids readily condense with glucose to form humin (Fig. 1). Humin, like lignin, is not affected by 72 per cent sulfuric acid and would therefore be indistinguishable from it in the analysis. Indeed, from the data it appears possible that true lignin as such is lost in the degradation and that the increase in apparent lignin is due solely to humin formation.

TABLE 6

Analysis of Nitrogenous Substances in the Two Varieties of Beets Used

Variety and treatment	Total N [1]	Amino N [1]	Protein N [1]	Ammonia N [1]	Amide N [1]
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
DR	2.22	.28	.90	.04	.21
DR-40	1.98	.38	.84	.04	.14
DR-50	2.02	.20	.87	.11	.20
DR-60	2.14	.18	.89	.09	.06
DR-70	2.26	.07	.60	.01	.04
DR-60op	2.01	.18	.81	.07	.16
DR-50-2wk	2.07	.30	.71	.01	.22
DR-50-4wk	2.05	.27	.72	.01	.14
DR-50-6wk	2.06	.27	.55	.13	.09
EC	2.04	.32	.79	.04	.16
EC-40	1.86	.27	.94	.06	.14
EC-50	1.88	.24	.73	.11	.08
EC-60	1.90	.07	.79	.08	.07
EC-70	2.05	.03	.70	.01	.06
EC-60op	1.75	.18	.69	.05	.10
EC-50-2wk	1.84	.33	.65	.01	.09
EC-50-4wk	1.85	.18	.70	.00	.09
EC-50-6wk	1.84	.18	.61	.05	.05

[1] Total N=per cent nitrogen by Kjeldahl; amino N=per cent nitrogen by ninhydrin reaction; protein N=per cent nitrogen by ninhydrin reaction after hydrolysis of protein—per cent nitrogen by ninhydrin reaction before hydrolysis; ammonia N=per cent ammonia nitrogen evolved upon heating in basic solution; amide N=per cent ammonia nitrogen after hydrolysis of solution remaining from ammonia N determination.

Were the glucose to follow solely this course of degradation the increase in the lignin fraction should be greater than the decrease in the total sugar (because of combination with nitrogenous substances, although it is conceivable that nonnitrogenous polymerization may occur). Although this is true of the DR's, it is not true of the EC's. Glucose therefore follows additional paths of degradation, and they may be numerous. In the case of beets the primary path is, nevertheless, via humin formation.

Cellulose is of interest in providing an additional reference base. Both varieties provide similar data—an initial decrease followed by an increase to a value exceeding the original. The initial decrease appears to be a reflection of the "binding" of water, which results in apparently diminished values of poorly hydrated substances, as the sugars and lignins, but increases values of pectic substances and proteins (Tables 4 and 5). The

subsequent increase in cellulose is presumably due to the diminution of organic matter by decarboxylation, deamination, etc. The pectic substances display a variability, not of an analytical nature (see Methods).

Nitrogenous Substances: The nitrogenous fraction as a whole is the second largest group present in red beets. A group analysis, by no means satisfactory, is presented (Table 6 and Fig. 2). Only one of the groups, protein, is related to particular substances although there is such a predominance of glutamine in the amide nitrogen group that the analysis is capable of correlation to a certain extent. The group designation of amino nitrogen is somewhat misleading, being properly α-amino acid nitrogen.

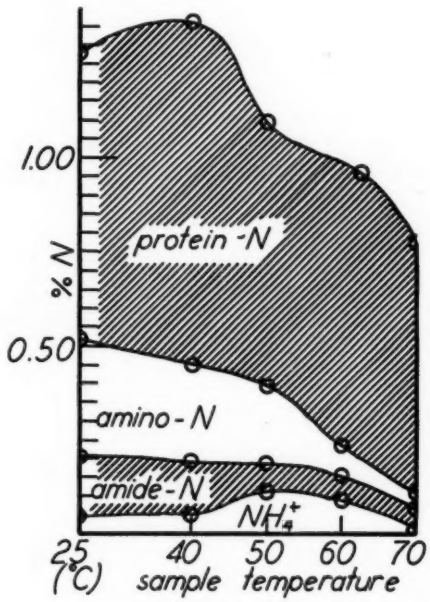

FIG. 2.

At least two other groups, pigment nitrogen and betaines, known to be present in significant amounts are not represented. In addition, beets are known to contain various alkaloids, a certain amount of lecithin, etc. The initial group fractions comprise somewhat more than half of the total (Kjeldahl) nitrogen, the final less than a third; these are, then, essentially labile fractions.

A perusal of the table brings out the following facts: (1) although the major groups all decrease with increasing degradation, (2) the total nitrogen, after a small initial decrease, actually increases to a value exceeding the original; (3) the variation in the protein fraction strongly resembles that in pectin, where an apparent diminution was interpreted as due to strong hydration. The decrease is, for reasons in addition to that of numerical decrease, ascribed to hydrolysis of protein.

Total nitrogen, as ash and cellulose, serves as a reference base. The initial decrease is due to the adsorption of water by strongly hygroscopic materials, as previously explained; similarly the increase is a reflection of the loss of organic matter (nonnitrogenous) so that the actual per cent of nitrogen increases. For reasons explained in the methodology, it cannot be interpreted as an increasing inability of the Kjeldahl method to analyze for nitrogen.

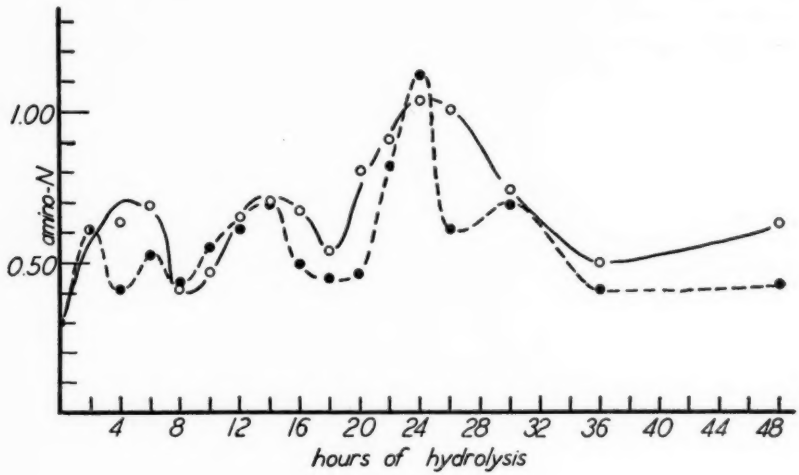

FIG. 3.

Among the most interesting aspects of the nitrogenous fraction was the variability in protein. This variability manifested itself in two diverse directions: first, that corresponding to the different degradational temperatures, and second, the time required to give maximal hydrolysis (measured by ninhydrin amino-acid determinations) with those different samples. In the latter case it was ascertained that whereas maximal yield was obtained in the control (25°C.) after 24 hours' hydrolysis, only 14 were required in the 70°C. sample. This indicates partial hydrolysis had occurred in the more degraded samples.

In the course of determination of maximal hydrolysis times, analyses indicated a series of maxima rather than the expected continually increasing amount of amino nitrogen (Fig. 3). This occurred presumably because of its rapid removal in some other process as humin formation. These maxima may be interpreted as an indication of the existence of a number (at least three) of major proteins. The assumption involved is that the different proteins hydrolyze at different rates, and the results indicate that at least three, and possibly five, major proteins exist within red-beet tissue. It is not improbable that under suitable conditions (e.g., the addition of sugars or other conjugable substances) this can be utilized as an analytical tool for the indication of the existence of multiple protein components.

Slightly indirect evidence for the continuous hydrolysis of protein during degradation is provided by the increase in the DR-40 sample of the amino-acid nitrogen. The loss of this material with increasing degradation cannot as yet be stated with certainty. The primary probabilities are (a) either a decarboxylation, or a deamination, has occurred so that the test as such is no longer effective although polymerization need not have occurred; or (b) the material has combined with aldehydes to form humins. In view of the observed rapid increase of humin with increasing degradation, the latter explanation is preferred [Gortner and Holm (1917)].

It is to be noted that ammonia nitrogen shows a maximum at 50°C., indicating its liberation (probably from amides) and reutilization.

As in the case of the sucrose hydrolysis, the nonenzymatic protein hydrolysis is of considerable significance. A number of nonspecific hydrolytic catalyses have been proposed in theory especially for inorganic substances, though it is doubtful whether the results of this type of study have any significance in normal tissue. This phenomenon appears to us to be related to a disorganized state and possibly to the extent and nature of capillaries produced in dehydration. The subject in general obviously requires additional investigation.

Among the problems which the authors believe require further investigation are the following:

(a) Quantitative gas-exchange measurements, as oxygen absorption and carbon dioxide evolution.

(b) Chemical nature of lignin changes, i.e., determination of nitrogen content of the various products, etc. This includes separation of true lignin from humin.

(c) Chemical structure of the pigments as well as completion of separation and purification; manner of breakdown.

(d) Basis of flavor changes.

SUMMARY

Aquothermal degradation of dehydrated red beets, at temperatures ranging from 40 to 70°C., was studied by analysis of changes in constituents which account for 90 to 91 per cent of the solid content. Determinations are given for non-reducing and reducing sugars, pectic substances, starch, cellulose, and lignin; nitrogenous constituents are total (Kjeldahl), amino acid, protein, ammonia, and amide nitrogen. Degradation results in hydrolysis of sucrose and probably combination of the resulting glucose with amino groups. The lack of reaction of fructose causes increases in the reducing sugars. Considerable hydrolysis of proteins occurs, and evidence is provided for the existence of at least three abundant proteins with differing rates of acid hydrolysis.

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THERMAL DEGRADATION OF DEHYDRATED BEETS
II. CHROMATOGRAPHIC SEPARATION OF RED BEET-ROOT PIGMENTS [1]

S. ARONOFF [2] AND E. M. ARONOFF
Department of Chemistry, University of Chicago, Chicago, Illinois

[Received for publication, March 1, 1947]

The beet pigments are distinguished by the fact that, although resembling anthocyanins in many of their reactions, they are nitrogenous. Pending identification of their structure, they are commonly referred to as "nitrogenous anthocyanins." Their known occurrence is limited to five plant orders—Cactales, Thymeleales, Lythrales, Chenopodiales (including beets), and Caryophyllales—and includes 25 species, Lawrence, Price, Robinson, and Robinson (1938).

The first work on the beet pigments appears to have been performed by Schudel (1918), working with Willstätter, who isolated the pigments as the picrates and, believing them to be a single, pure compound, adduced an empirical formula. Further work on isolation, purification, and attempts at delineation of structure have been performed by a number of workers: Ainley and Robinson (1937); Chmielewska (1938); Pucher, Curtis, and Vickery (1938); Robinson and Robinson (1932); and Schmidt (1937). Little is known concerning the nature of the nitrogens, and although Schmidt (1937) has identified one of the two as a Van Slyke amino nitrogen, this has been denied by Pucher, Curtis, and Vickery (1938). The only common finding is the presence of a hydrolyzable glucose, the original pigments existing as glucosides. Kozlowski (1921) has obtained a colorless chromogen from sugar beets, and by similar procedure, a yellow compound from red beets, which on oxidation in sulfuric acid and MnO_2 gives a series of pigments with spectra similar to beet pigments. The chromogens are referred to as having the properties of saponins. Chmielewska (1938) has reported the separation of beet pigments chromatographically on alumina into red and orange components, which she identifies as the glucoside and the aglucoside, but it is not clear whether this orange represented a concentrate of the yellow or was another pigment.

We have found that the beet pigments may be separated chromatographically into at least 11 bands, J and K (Fig. 1) appearing on further attempts at purification to be multiple and therefore causing doubt as to the exact number. Furthermore there appears to be a vague distribution of yellow material between E and F.

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[1] The subject matter of this paper was undertaken in co-operation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

[2] Present address: University of California, Radiation Laboratory, Berkeley 4, California.

Chromatography is most successful on a talc-siliceous earth mixture in 3:1 volume ratio (Mallinckrodt's purified talc and Hyflo-super-cel were used). The beet pigments are characterized by insolubility in other than aqueous solution, and water alone is used both as solvent and eluant. Adsorption may be performed either directly from beet sap or from a readily prepared pigment concentrate (described below) with identical results. The general appearance of the column is shown (Fig. 1). No

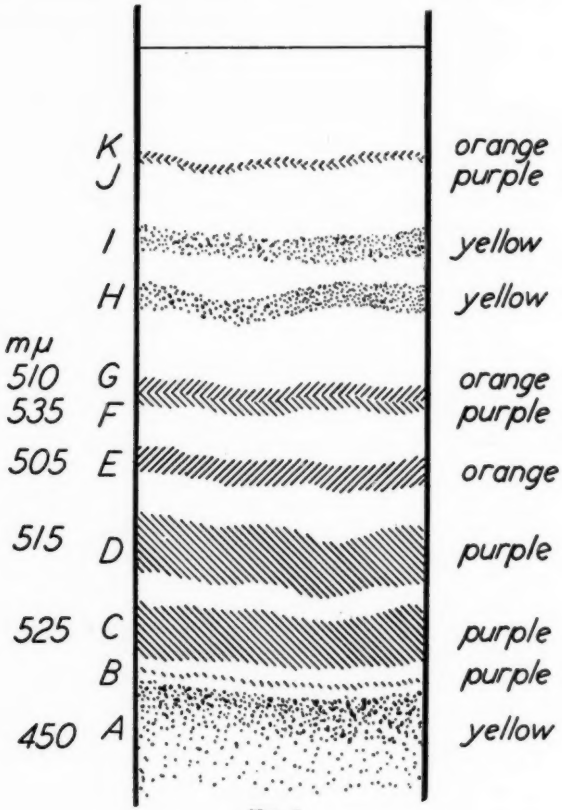

FIG. 1.

differential eluant has been found, and fractionation of the pigments is at present accomplished by extrusion of the column, mechanical separation of the various bands, and solution of the adsorbed pigments in water (alcohol aids in removal of the material from talc).[3] Further procedure

[3] The necessity for extrusion and the sole use of water has permitted and indeed required the use of a simplified type of column (Fig. 2). The constriction in the ordinary ground-joint columns makes it impossible to extrude the columns without distortion. Furthermore, the use of water appears to require more even packing of columns than in nonaqueous techniques, and tampers have been modified by the attachment of a rubber terminus which expands against the sides while packing.

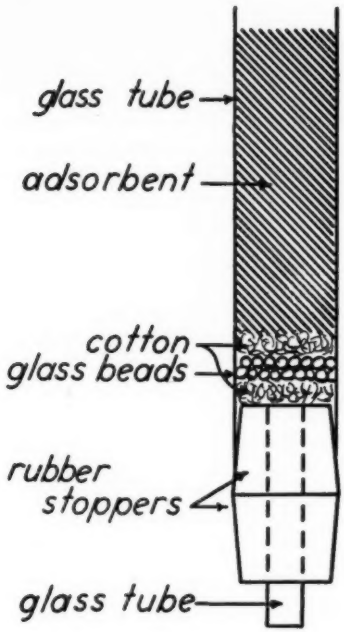
glass tube
adsorbent
cotton
glass beads
rubber
stoppers
glass tube

FIG. 2.

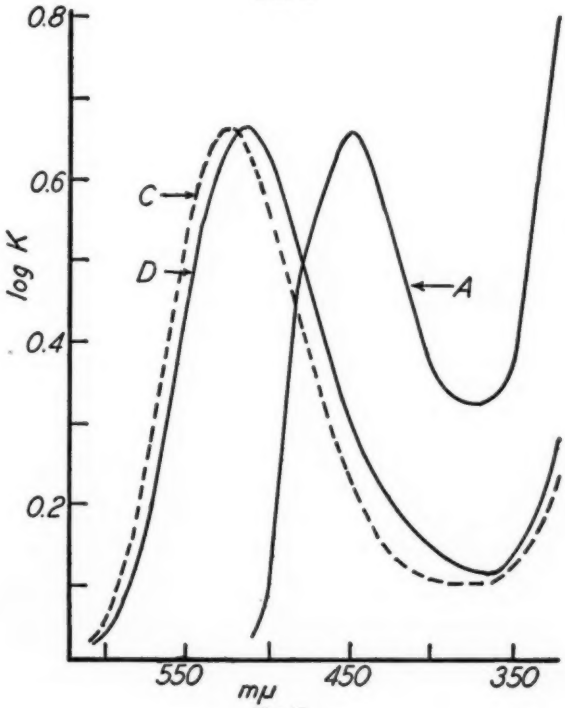
0.8
0.6
0.4
0.2
log K
C
D
A
550
450
350
mμ

FIG. 3.

is a matter of choice, since the pigments are all precipitable as lead or mercuric salts which can then be decomposed and the pigments regained. Their insolubilities in solvents other than water permit their complete separation from aqueous or acido-aqueous solution by the addition of acetone or alcohol-ether. When precipitated from water alone, the material is extremely hygroscopic. For our purposes, resort was made to lyophilization of the various fractions, the pigments being rechromatographed until complete resolution was obtained for purposes of running absorption spectra.

A pigment concentrate is readily prepared in the following manner. Two volumes of cold acetone are added to one volume of beet sap, and flocculation of pectinous substances is permitted to occur. The solution is filtered, and to the filtrate twice the volume of acetone is again added, the mixture being allowed to stand overnight in an ice chest. The pigment concentrate separates out as a dark, red-black, oily mass on the bottom of the vessel. The upper solution is readily decanted and is suitable for recovery of acetone. Further purification of the pigment concentrate may be made by adding twice the volume of 95 per cent ethanol to an aqueous solution of the concentrate.

Absorption spectra of various components, obtained with a Beckman spectrophotometer, are as shown (Figs. 3, 4, and 5).

It is of interest to note that all the pigments display a similarity of spectra, having not only a single band in the visible with a half-width of approximately 50-60 mμ. but a second band in the ultra-violet. In the case of the lower group the secondary maximum occurs at ca. 260 mμ., while in the middle it occurs near 300.

It was shown chromatographically that the difference in color between the two beet varieties used (Egyptian Crosby and Detroit Red) was not due to the absence of any pigment from the lighter colored Crosby but merely a total diminution.

The primary component in both varieties is yellow pigment A which, following the suggestion of Pucher, Curtis, and Vickery (1938), can be removed in large measure by preliminary extraction of beet tissue with hot alcohol. Preliminary attempts at identification of the yellow pigment indicate that it is not a member of the flavone group.

Fractions C and D are the primary purple components, although orange E attains considerable proportion, and the F (purple) and B (orange) above it are by no means inconsiderable.

In thermally degraded beets two processes occur: a gradual loss of all the pigments and the formation of brownish material. Chromatography separates the contaminant, which is strongly adsorbed on the upper portion of the column, the other pigments preserving the same order; the general appearance of the column is shown (Fig. 6). Considerable destruction of the yellow A has occurred, the band at 450 mμ. having almost entirely disappeared. Bands C, D, and E are grossly diminished, and the primary pigments remaining are F and G.

The lability of the lower components is attested by the fact that both C and D (solid, purified) standing in a glass-stoppered vessel (room tem-

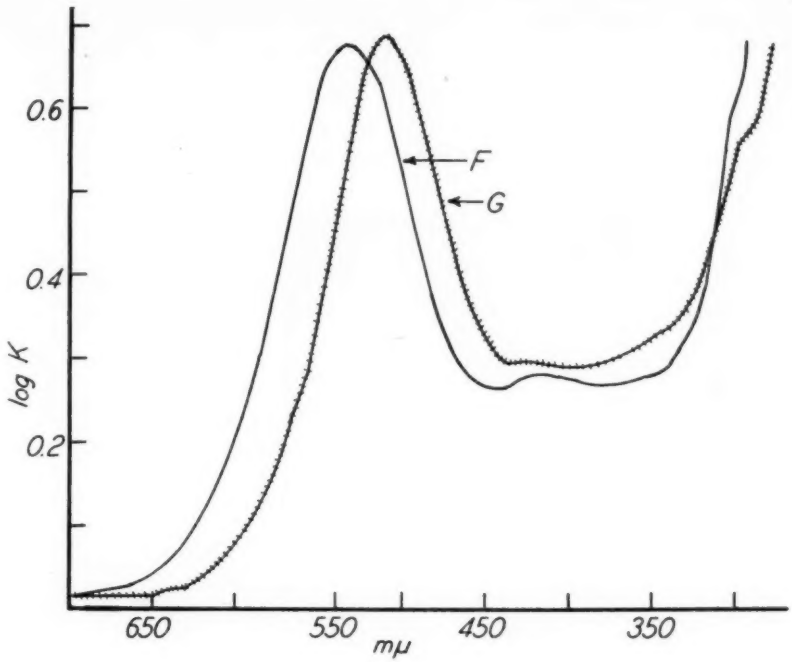
0.6
0.4
0.2
log K
F
G
650
550
450
350
mμ

FIG. 4.

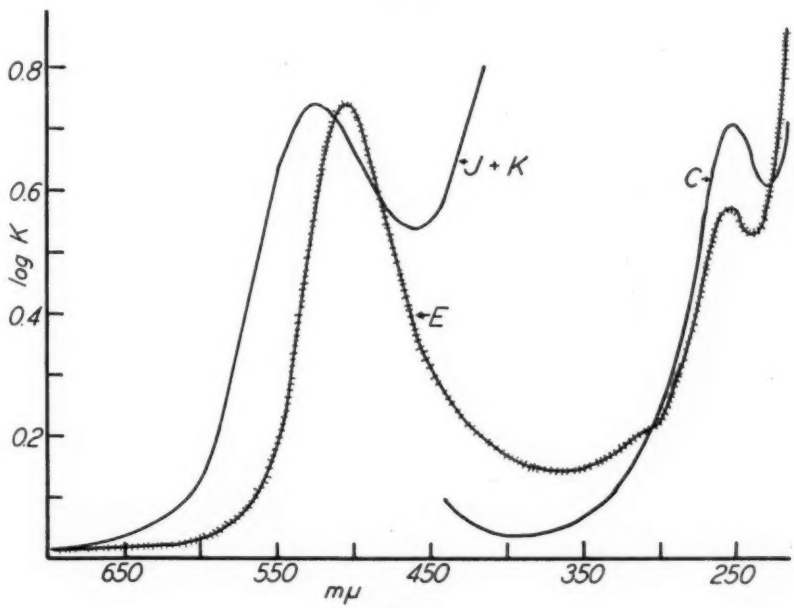
0.8
0.6
0.4
0.2
log K
J + K
C
E
650
550
450
350
250
mμ

FIG. 5.

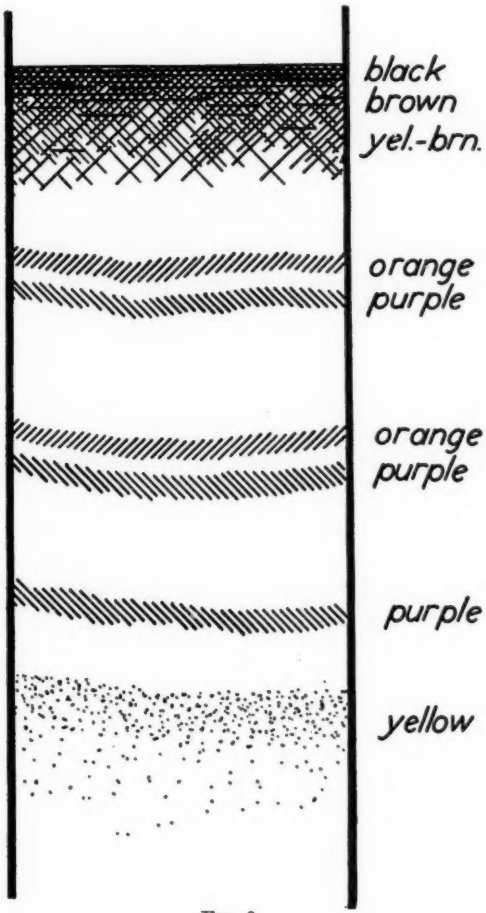
black
brown
yel.-brn.
orange
purple
orange
purple
purple
yellow

FIG. 6.

perature, no desiccant) turned yellow-orange after two to three days. Color could not be regenerated by either oxidative or reductive procedures. Components J and K were extraordinarily stable, and it thus appears that the stability of the pigments increases the higher they are on the column. Aqueous solutions of all components, kept in a cold room [2 to 3°C.(35.6 to 37.4°F.)] appeared to be more stable than the solids.

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ESTERS OF VANILLIC ACID AS SPORE-CONTROLLING AGENTS

FRED R. EVANS AND HAROLD R. CURRAN
Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture

[Received for publication, March 17, 1947]

The control of resistant bacterial spores in food and other perishable materials low in acid presents one of the most difficult of preservation problems. Heating under steam pressure is not always practicable and few chemical substances in permissible concentrations are effective or entirely suitable. The esters of parahydroxy benzoic acid are widely used as general preservatives and in Europe have found application in the conservation of certain food materials. Certain analogs of parahydroxy benzoic acid—the vanillic acid esters—have been shown to be inhibitors of glucose fermentation by yeast, Sabalitschka and Tietz (1931); and recently these compounds have received emphasis as potentially useful preservatives of bread, cheese spreads, pickled herring fillets, and vegetable and fruit juices, Pearl (1945) and Pearl and McCoy (1945). Of particular interest in the latter report was the observation that certain of the vanillic acid esters in low concentrations inhibited the development of a heat-resistant, spore-forming species (*Bacillus mycoides*) when the latter was cultivated on nutrient agar plates in the presence of the reagent. The inoculum was so prepared as to insure the presence of viable spores. The possible utility of these compounds for the preservation of substances low in acid and containing spores prompted us to study their spore-controlling activity on bacteria of widely differing species. The results are presented in this paper.

EXPERIMENTAL PROCEDURE

Four vanillates[1]—ethyl, n-propyl, n-butyl, and isobutyl—were used. Because of their limited solubility in water the reagents were dissolved in a slight excess of normal sodium hydroxide and distilled water dilutions of these stock solutions as their sodium salts were used to provide the concentrations desired.

The spores of the aerobic species were produced on nutrient agar slopes contained in large dilution bottles, those of *Clostridium perfringens* on nutrient agar slopes contained in tubes incubated anaerobically using alkaline pyrogallol, those of *Clostridium botulinum*, and No. 3679 in deep brain cultures. Except for the latter two, which were inoculated directly, all of the spore crops were thoroughly washed before use. The washed spores were seeded uniformly into sterile (autoclaved) skim milk (10 ml. per tube) to yield a concentration of 100,000 viable spores per milliliter. The samples were then heated at 95°C.(203°F.) for 15 minutes, cooled, and the reagents added and thoroughly mixed with the inoculated milk (final pH 6.6). The temperature and period of storage were as indicated. At the higher temperature of incubation sterile rubber stoppers were inserted over the cotton plugs to retard evaporation.

[1]Obtained from Dr. Irwin A. Pearl, The Institute of Paper Chemistry, Appleton, Wisconsin.

Thirty-four cultures comprising 16 species were studied. These included 20 mesophilic aerobes, 10 thermophilic aerobes, and four mesophilic anaerobes. The final concentration of spores was greatly in excess of that normally encountered in food and nonfood materials and the results therefore afford a margin of safety for calculation purposes. Weekly observations for evidences of growth were made for at least three months; the results are presented (Table 1).

Isobutyl vanillate was uniformly effective in preventing spoilage but only in 0.15 per cent concentration (Table 1). N-butyl vanillate was only slightly less effective but had little inhibiting effect upon *Cl. perfringens*. N-propyl vanillate in 0.15 per cent concentration afforded protection against most of the aerobes but was ineffective against the anaerobes, particularly *Cl. perfringens*. N-propyl in the lower concentration and ethyl in the higher concentration rarely afforded complete protection.

Prevention of visible growth as it occurred in these tests was the result of sporistatic rather than sporicidal activity of the vanillates since incubation of nutrient agar plates inoculated with suitably diluted portions of the stored samples yielded large numbers of colonies. Storage temperatures were for the most part at or near the optimum for the culture concerned; from this it may be presumed that lower storage temperatures would materially prolong the sporistatic effect.

Although isobutyl vanillate in 0.15 per cent concentration proved to be an effective sporistatic agent in milk, the flavor imparted by this concentration of the compound would render it unacceptable for human consumption. The impairment in flavor so produced is considerably greater than that caused by a comparable concentration of sodium benzoate. In more strongly flavored milk products, such as cheese spreads, the ethyl and n-propyl vanillates may have value in certain circumstances, particularly as mold inhibitors, Pearl and McCoy (1945). Other possible applications in the preservation of food products have been indicated by these authors.

Animal toxicity of ethyl vanillate is now under investigation at the Kettering Laboratory of Applied Physiology using rabbits, guinea pigs, and rats as test animals. These studies [2] indicate that ethyl vanillate is no more and possibly less toxic than sodium benzoate or benzoic acid (sodium benzoate is permitted as a preservative agent in many food materials in concentrations up to 0.1 per cent) in its effect upon laboratory animals. Continued feeding to rats of ethyl vanillate up to two per cent of the total food intake has no apparent effect upon their weight, mortality, and fertility over an 18-months period. When orally administered in olive oil to rabbits or rats the lethal doses of sodium benzoate and ethyl vanillate are comparable for each animal, but when orally administered in aqueous suspensions the vanillate is much less toxic than the sodium benzoate.

Although no systematic study was made of the sporistatic action of sodium benzoate, a few observations were made in order to provide some basis for comparison with the vanillates. The action of sodium benzoate in 0.15 per cent concentration in milk was tested against the spores of

[2] Private communication from Dr. Henry W. Ryder.

TABLE 1

Sporistatic Activity of Esters of Vanillic Acid in Milk Inoculated With Bacterial Spores and Stored for a Minimum of Three Months

Name and number of organism		Temperature of storage	Inoculated control with no vanillate	Vanillates							
				Ethyl		N-propyl		N-butyl		Isobutyl	
				0.10	0.15	0.10	0.15	0.10	0.15	0.10	0.15
		°C.									
B. laterosporus	9141	30	S14	U[1]	U	U	U	U	U	U	U
B. laterosporus	8248	30	S55	U	U	U	U	U	U	U	U
B. sphaericus	7063	30	S30	S40[1]	U	S73	U	U	S69	U	U
B. macerans	7069	30	S30	S73	U	U	U	U	U	U	U
B. pumilus	7061	30	S9	S9	S83	S28	U	S13	U	U	U
B. polymyxa	8240	30	S5	S9	S13	S9	U	S13	U	S20	U
B. brevis	PennS	30	S15	S100	U	U	U	U	U	U	U
B. brevis	8185	30	S15	S31	U	U	U	U	U	U	U
B. brevis	8186	30	S15	S31	U	U	U	U	U	U	U
B. megatherium	234	30	S14	S22	U	U	U	U	U	U	U
B. megatherium	696	30	S5	S9	S24	S20	U	S24	U	U	U
B. cereus	202	30	S5	S12	S28	S99	U	U	U	U	U
B. cereus	369	30	S5	S12	S30	S28	U	S28	U	S73	U
B. cereus	401	30	S6	S15	S47	S62	U	U	U	S62	U
B. mycoides	6462	30	S6	S35	U	U	U	U	U	U	U
B. metiens		30	S6	S9	S40	S47	U	S47	U	U	U
B. subtilis	6051	30	S5	S9	S13	S13	S13	S13	S20	S13	U
B. subtilis	6	30	S5	S5	S14	S22	S47	S40	U	S40	U
B. aterrimus	230	30	S7	S7	S12	S20	S26	S22	U	S20	U
B. niger	6454	30	S7	S12	S21	S28	U	S33	U	S40	U
B. calidolactis	11	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	1518	52	S14	S14	S14	S60	U	U	U	U	U
B. stearothermophilus	7028	52	S14	S14	S14	S60	U	U	U	U	U
B. stearothermophilus	26	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	1503	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	C1P4	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	C2P3	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	07	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	M17	52	S14	S14	S14	S14	U	U	U	U	U
B. stearothermophilus	1A	52	S14	S14	U	U	U	U	U	U	U
Cl. botulinum	62A	30	S15	S19	S22	S22	S55	S55	S130	S130	U
Cl. sp.	3679	30	S6	S6	S19	S25	S130	S31	U	S50	U
Cl. perfringens	846	37	S1	S2	S2	S2	S2	S2	S2	S2	U
Cl. perfringens	3526	37	S1	S2	S2	S2	S2	S2	S11	U	U

[1] S=visible growth; U=no visible growth; numbers indicate period in days to first observed growth.

10 cultures, including both aerobic and anaerobic species and under conditions comparable to those used in the study of vanillates. In this experiment most of the test samples spoiled within three months although visible evidence of growth was usually deferred for some time. Thus in contrast to isobutyl vanillate, sodium benzoate in 0.15 per cent concentration is not an efficient spore-controlling agent in substrates low in acid.

DISCUSSION

Prolonged preserving action of vanillates, as observed in this study, resulted probably from the inability of the spores to germinate in the concentration of reagent used, although the possibility of slow germination followed by inhibition of vegetative development is not excluded. Whether nonsporing bacteria would be as readily controllable by isobutyl vanillate as are spores is not known, but the ease with which the former can be destroyed by relatively mild heating renders the question of no great significance, except where the application of heat is impracticable.

A precedent for the limited acceptance of ethyl vanillate as a food preservative was established during World War II when the United States Food and Drug Administration agreed to consider its use in amounts up to 0.1 per cent when it could be demonstrated that such use would permit the delivery of acceptable food products to the armed forces. The greater animal toxicity of benzoate compared with that of ethyl vanillate in aqueous substrates has been mentioned. In view of the prolonged sporistatic activity of the isobutyl vanillate animal toxicity data on the latter compound would be of considerable interest.

SUMMARY

The spore-controlling action of four vanillic acid esters was observed in milk containing 0.10 and 0.15 per cent of the reagents. Thirty-four cultures, comprising 20 mesophilic aerobes, 10 thermophilic aerobes, and four mesophilic anaerobes, were used. The temperature of storage was 30, 37, or 52°C.(86, 98.6, or 125.6°F.); the time of observation was three to four months.

Isobutyl vanillate was the most efficient preserving agent. In 0.15 per cent concentration all the samples remained visibly unchanged over the storage period. N-butyl vanillate in comparable concentration was somewhat less effective, particularly against the anaerobes (*Cl. botulinum* and *Cl. perfringens*). Ethyl vanillate at the higher level delayed, but rarely prevented, growth. Sodium benzoate in 0.15 per cent concentration in milk is relatively ineffective as a spore-inhibiting agent. However, while 0.15 per cent sodium benzoate produces very little change in the flavor of milk, a like amount of any of the vanillates produces a distinctly objectionable flavor.

The action of the reagents in all cultures was primarily sporistatic.

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SABALITSCHKA, T., AND TIETZ, H., 1931. Synthetische Studien über die Beziehung zwischen chemischer Konstituton und antmikrobischer Wirkung XI. Arch. Pharm. 269, 545.

Cl. perfringens	3526	37	S1	S2	S2	S2	S2	S2	S11	U	U
Cl. perfringens											

[1] S=visible growth; U=no visible growth; numbers indicate period in days to first observed growth.

A FURTHER INQUIRY REGARDING EFFECT OF ELECTROLYTES ON SWELLING OF CEREAL STARCHES [1,2]

R. H. HARRIS AND ORVILLE BANASIK [3]
North Dakota Agricultural Experiment Station and School of Chemical Technology, North Dakota Agricultural College, Fargo, North Dakota

[Received for publication, March 24, 1947]

Utilization of starch is directly dependent upon its colloidal properties, such as viscosity, plasticity, gel strength, etc., since starch is principally employed in the form of a sol, paste, or gel. These properties are associated with the hydration or swelling power of the starch, and their determination is an indirect measure of starch hydration. Certain weaknesses, however, are inherent in methods used for assessing hydration from viscosity data. Changes in starch-paste consistency may also be detected by instruments which show alterations in the resistance of the paste to mixing during gelatinization.

Harris and Jesperson (1946a) briefly summarized a few of the many contributions to the indirect determination of starch swelling and described an alternative method which attempts to measure directly this starch property. This method entails heating one per cent starch pastes for five minutes in centrifuge tubes in a water bath. The swell was determined by three procedures as follows: (1) The centrifuge tube was weighed before adding the required quantity of starch, then reweighed after centrifuging and draining off the supernatant liquid. The gain in weight was attributed to the water absorbed or swell of the starch. (2) The volume of the gelatinized starch was determined following centrifugation, using graduated centrifuge tubes. Both methods of determination were employed on the same sample. (3) Samples of cooked starch were removed from the central portion of the residue to obviate securing free water in the material, and the moisture content was determined by drying. This method appeared to be less subject to error than the other two and was used for further swelling power investigations although it was the more tedious. The three methods all measured water in the starch granules, water absorbed on them, and interstitial water between them. The results obtained by the three methods were very significantly related. "Swelling power" and "swell" are used synonymously in this work, and imply the quantity of water in grams taken up by one gram of dry starch.

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[1] Published with the approval of the director of North Dakota Agricultural Experiment Station.

[2] A further report upon researches conducted at the North Dakota Agricultural Experiment Station under Adams Project 24, "A study of certain wheaten starch properties with emphasis upon those of a physical nature in respect to the effect of maturity, variety, and environment."

[3] Research assistant, Department of Cereal Technology, North Dakota Agricultural Experiment Station, and graduate student, School of Chemical Technology, North Dakota Agricultural College.

The investigations reported in this paper are concerned with the effects of different electrolytes on the swelling power of corn and wheat starch which had been prepared by various procedures. The method employed for assessing the swell was the third one in the description of former procedures used in this laboratory.

EXPERIMENTAL PROCEDURE

Preparation of Starches: One sample of commercial cornstarch was employed, while the other starches were prepared in the laboratory from hard red spring wheat flour. One of these was separated from a stiff dough by washing with 0.1 per cent sodium phosphate solution of approximately pH 6.8. Another was isolated by treatment with 0.125 N NaOH at 5°C.(41°F.) for 18 to 20 hours with occasional stirring. A third wheat starch was prepared in a similar manner with 0.122 N H_2SO_3. These three wheat starches were separated from liquid by centrifugation and washed a number of times, first with distilled and finally with redistilled water. They were then dried at room temperature under a fan, and passed once through a small grinder to break up lumps.

TABLE 1

Comparative Data From Starches Used[1]

Description of starch	Moisture	Protein	Ash	pH	Swell
	pct.	*pct.*	*pct.*		*gm./gm.*
Wheat starch, prepared with 0.125 N NaOH	11.9	.06	.31	9.2	32.1
Commercial corn starch	9.2	.33	.10	6.6	29.4
Wheat starch, treated with 0.007 N Cl_2 soln.	9.3	.20	.09	4.9	27.1
Wheat starch, treated with 0.014 N Cl_2 soln.	9.3	.20	.09	4.7	26.6
Wheat starch, prepared with 0.122 N H_2SO_3 soln.	9.0	.25	.09	5.3	18.4
Wheat starch, prepared by washing with water	14.3	.20	.09	6.3	14.6

[1]Arranged in order of decreasing swell when cooked in redistilled water at 95°C.(203°F.).

Two additional starches were derived from the starch originally washed from dough by treatment with 0.007 and 0.014 N solution of chlorine in a similar manner to those separated from flour by NaOH and H_2SO_3 except that starch rather than flour was used as source material. Analytical data collected from the five starches are shown (Table 1); it will be noted that treatment with 0.125 N NaOH significantly raised the pH, while 0.122 N H_2SO_3 lowered it. Similarly, exposing the starch to chlorine lowered the pH of the original starch. There was a marked disparity among the samples in swell when gelatinized in redistilled water at 95°C.(203°F.). There appeared to be a direct relation between pH and swell.

Methods: Moisture, nitrogen, and ash determinations were made essentially by the procedures given in "Cereal Laboratory Methods," fourth edition (1941). The pH measurements were secured by the technique described by Harris and Jesperson (1946). The concentration of chloride ion in the water used for chlorinating the starch was determined by the

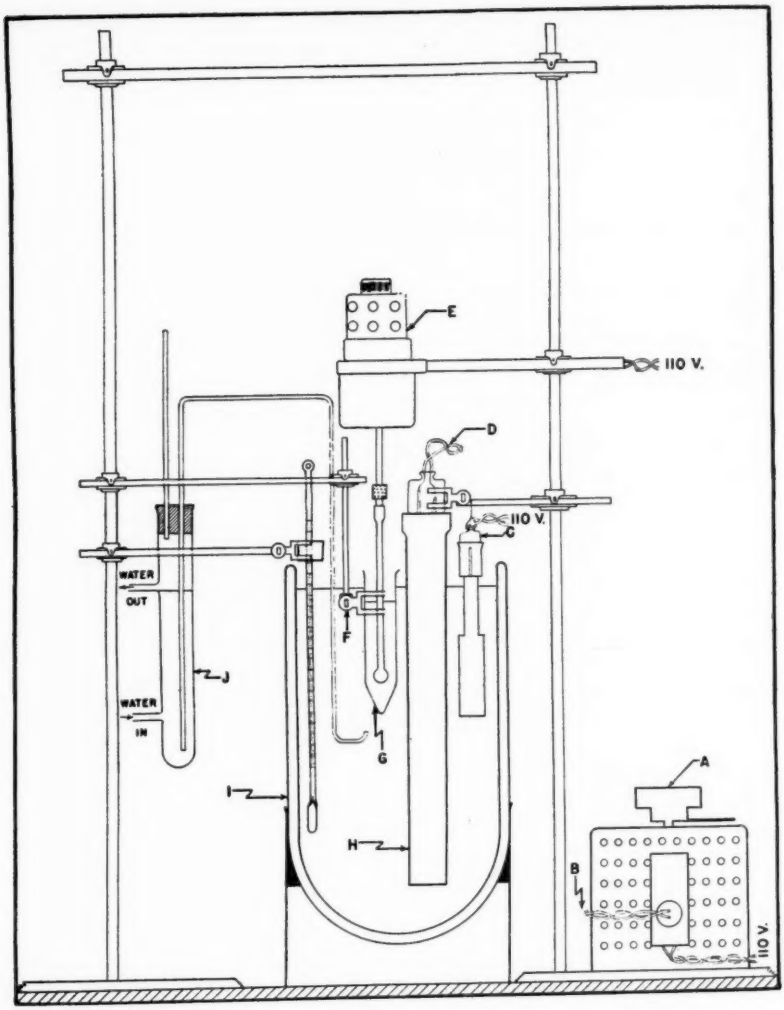

FIG. 1. Line drawing of starch cooker and accessories.

A. Variac to regulate line voltage to knife-blade heater.
B. Line from variac to heater.
C. Auxiliary for raising bath temperature quickly.
D. Line to variac.
E. Stirrer motor, connected directly to stirrer.
F. Adjustable clamp for 50-ml. centrifuge tube.
G. Centrifuge tube positioned in bath.
H. Knife-blade heater.
I. Double-wall Dewar flask.
J. Constant water-level device which allows inflow of water to replace evaporation losses during operation of the cooker. [Laycroft, Norman E., Chemist-Analyst 35, (2) 47, (1946).]

method of Mohr,[4] which employs standard $AgNO_3$ for titration, with K_2CrO_4 as indicator. The starches were cooked in a double-wall Dewar flask fitted with suitable heaters and a device for maintaining a constant water level in the vessel (Fig. 1). The heat intensity was regulated by altering the voltage delivered to the heater with a variable resistance. This set-up materially reduced slight variations in temperature which had been noted when using the apparatus described by Harris and Jesperson (1946a). Many of the determinations of swelling power were made in triplicate and the remainder were in duplicate. The procedure was essentially that described by Harris and Jesperson with minor deviations. For example, in the present investigation, 0.001 N solutions of the reagents were made up and added to the starch immediately before cooking rather than using four ml. of 0.01 N solution and diluting to 40 ml. in the centrifuge tube. The gelatinized starch was dried overnight in an electric oven held at 105°C.(221°F.). Aluminum moisture dishes were employed for drying in lieu of the 30-ml. ash capsules formerly used. The aluminum dishes had a greater diameter than the capsules and facilitated drying. The loss in weight of the samples yields directly the quantity of water associated with the cooked starch.

DISCUSSION OF RESULTS

The more important results secured from this investigation are presented in the form of diagrams rather than tables of precise values because of the difficulty of quantitatively duplicating the values in other laboratories. The trends are also more easily discerned from figures. There is little doubt that the same general trends would be established by similar studies employing comparable methods. The observations obtained have been entered in the figures, and those interested can quite easily transfer the values to a table if they so desire.

Effect of Method of Preparation: Wheat starch prepared by treating flour with 0.125 N NaOH and 0.122 N H_2SO_3, as well as starch from the same flour exposed to 0.014 and 0.007 N solutions of chlorine in redistilled water, were compared with starch washed from the flour dough by 0.1 per cent sodium phosphate solutions of pH 6.8 (Fig. 2). These starches were all cooked in redistilled water at temperatures ranging from 60 to 95°C.(140 to 203°F.). The starch isolated with NaOH showed the greatest swelling power above 70°C.(158°F.). This is probably due to replacement of cations in the starch by sodium ion, as postulated by Ripperton (1931), with resultant effect on swell. Preparation with H_2SO_3 exerted the least influence on swell of any of the agents used, only increasing swell slightly above that of the control at 95°C. At equivalent concentration the action of H_2SO_3 is very much less than that of NaOH. It also appears to be less than that of H_2SO_4 and HCl, probably owing to the greater H-ion activity of the latter acids.

The two samples exposed to the action of chlorine in different concentrations gave interesting results. Although the stronger dosage of reagent

[4] Elementary Quantitative Analysis. Third edition (1940). H. H. Willard and N. H. Furman. D. Van Nostrand Co. Inc., New York, N. Y.

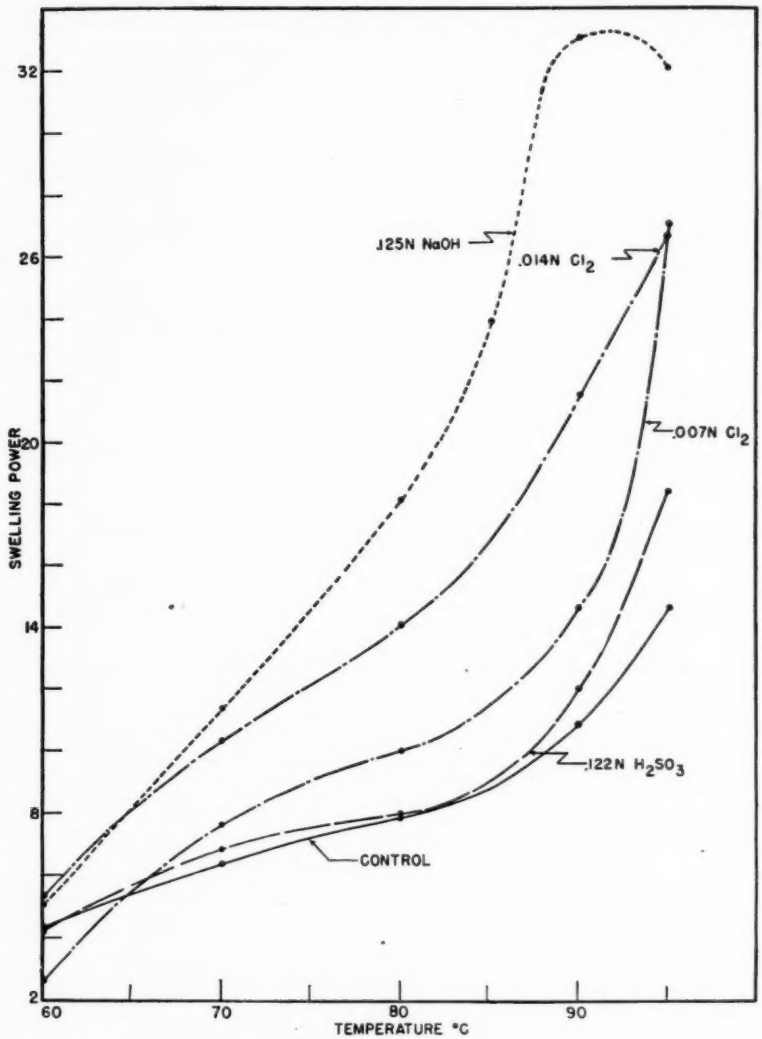

FIG. 2. Comparative effects on swelling power of various methods of preparing or treating wheat starch before cooking. Control starch washed from flour-water dough; cooking performed in redistilled water.

produced the greater swell until 90°C. was reached, the two curves intersect at 95°C. owing to the rapid increase in swell above 90°C. of the starch with the lighter treatment. Thus by heating to 95°C. a starch treated with a comparatively low concentration of chlorine, the swelling power would equal that of a starch exposed to twice the chlorine concentration. The principal effect of heavier chlorine dosage lies in increasing the swell at lower temperatures. The 0.14 N dosage did not give as much swell as the starch prepared with NaOH, but the concentration of the latter reagent was almost ten times that of the chlorine. There is little doubt that higher chlorine concentrations would increase swelling power and surpass the

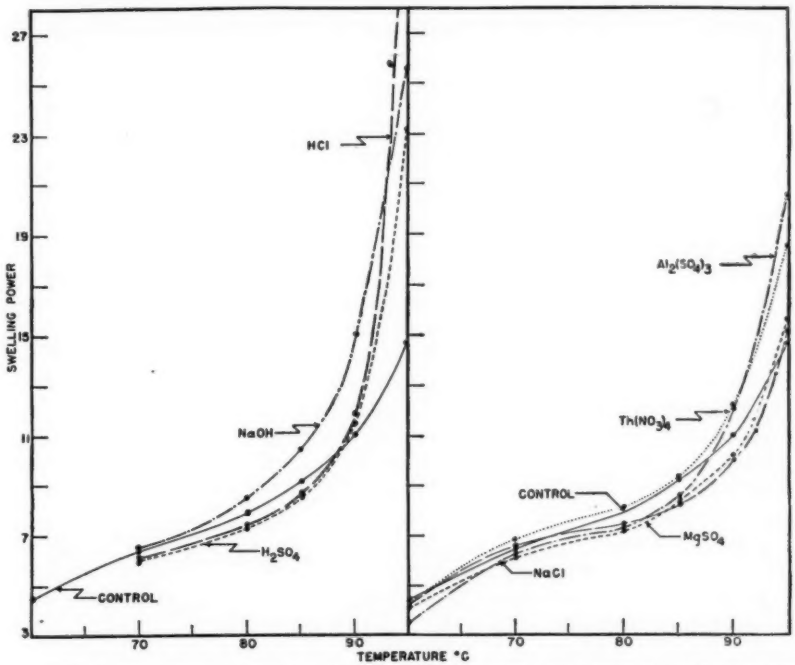

FIG. 3. The curves on the left show the effect on swelling power of cooking wheat starch with acid and alkali. The curves on the right represent the effect on swelling power of cooking wheat starch with solutions containing cations of different valencies. The control for both experiments was the same starch cooked in redistilled water. The starch was prepared by washing from a flour dough without chemical treatment.

effect of NaOH, provided the concentrations of the solutions were approximately the same. The explanation of the manner in which chlorine affects swell may lie in its effect on water with the formation of H ion, which in turn acts on the starch to increase the swell. However, this cannot be the only reason because the swell induced by chlorine is very much greater than that brought about by H_2SO_4 or HCl (Fig. 3). These results agree with industrial procedure in which chlorine gas is employed to increase the consistency of starch.

Effect of Cooking With Electrolytes: Studies previously reported by Harris and Jesperson (1946, 1946a) on the influence of ions on swell were enlarged, employing solutions of NaOH, HCl, H_2SO_4, NaCl, $MgSO_4$, $Al_2(SO_4)_3$, and $Th(NO_3)_4$ (Fig. 3). The effects of alkali and acid had not been studied in the previous investigations, and more information on the action of the inorganic ions was felt to be desirable. Section A on the left (Fig. 3) represents the effect of alkali and acid in the cooking solution on the swelling power of starch prepared by washing from dough without chemical treatment. There was little effect by the acids until a temperature of 90°C. was reached, when the swell was greatly accelerated. HCl had apparently a greater influence than H_2SO_4 at relatively high temperature. NaOH raised the swell even at 70°C. and the effect increased with rise in temperature but was less at 95°C. than the swell induced by HCl. It is evident from the results that pH is not the only factor active in determining swell because the pH of the cooking medium with NaOH would be much higher than that of the acid treatments.

Section B (Fig. 3) shows the effects of the various salts on starch swell. The changes from the control cooked in water are not so extreme as in A, but are no doubt significant for $Al_2(SO_4)_3$ and $Th(NO_3)_4$. The latter salts apparently increase the swell above 90°C., as compared with the control, while NaCl and $MgSO_4$ tend to decrease the swell until 95°C. is reached. These results are somewhat at variance with those reported by Harris and Jesperson (1946) but the method employed in the previous study used 98°C.(208.4°F.) as the cooking temperature, and the method of determining starch swell was quite different. It seems evident from the present data that the higher cation valencies may tend to increase the swell of cereal starch prepared without chemical treatment, but further work is required to settle this point. It appears that the H and OH ions are more effective in increasing the swell of untreated wheat starch than the other ions.

Cornstarch Swell: Commercial cornstarch was cooked with solutions of four electrolytes with different cation valencies (Fig. 4). NaCl and $MgSO_4$ markedly depressed the swell as compared with the control from the commencement of heating (70°C.). $Al_2(SO_4)_3$, on the other hand, did not change the swell greatly until 95°C. was reached, when the salt tended to depress swell. The effect of $Th(NO_3)_4$ was to increase swelling power through the entire heating range. The influence of the cation valency appeared to be quite marked in the study, decreasing the swell at valencies 1 and 2 for all temperatures, exerting little effect when the valency was 3, except at temperatures above 90°C. when it somewhat resembled 1 and 2, and increasing the swell when the valency was 4.

Effect of Cooking Solution on Swell of Starch Prepared With NaOH: The starch prepared by treatment with 0.125 N NaOH in the cold was cooked with solutions of H_2SO_4, NaCl, $MgSO_4$, and $Al_2(SO_4)_3$ (Fig. 5). The presence of the acid decreased swell extremely, owing very likely to the effect of the H ion. The sulfate ion apparently has little or no action since there were three sulphates included in this experiment and all gave widely different results. The cause of this disparity must therefore lie in the

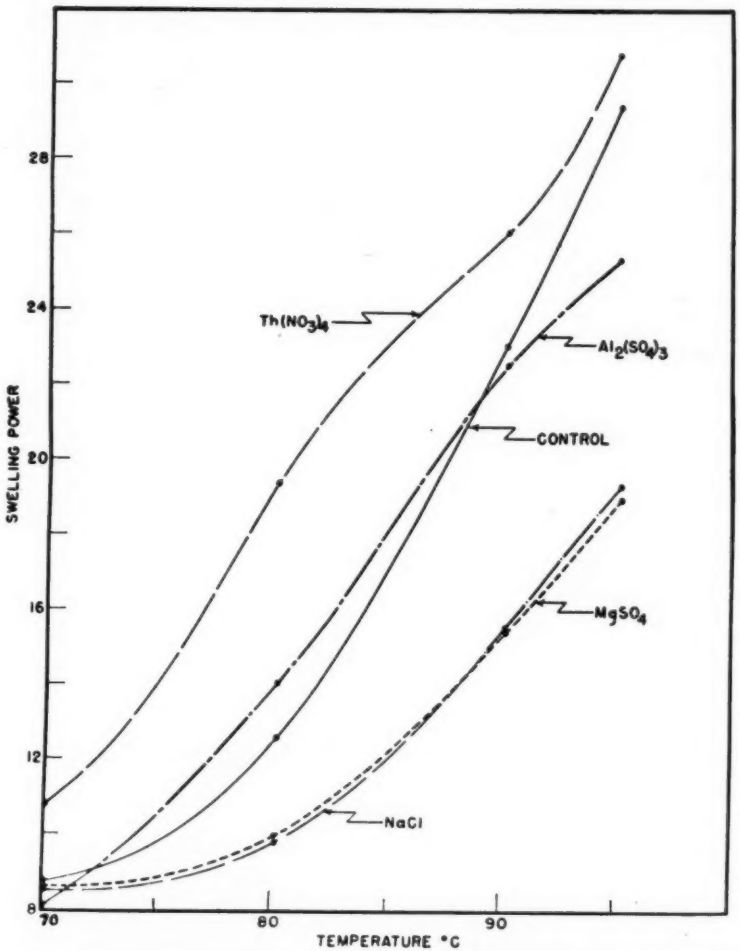

FIG. 4. Effect on swelling power of cooking commercial cornstarch with solutions containing cations of different valencies. The control was cooked in redistilled water.

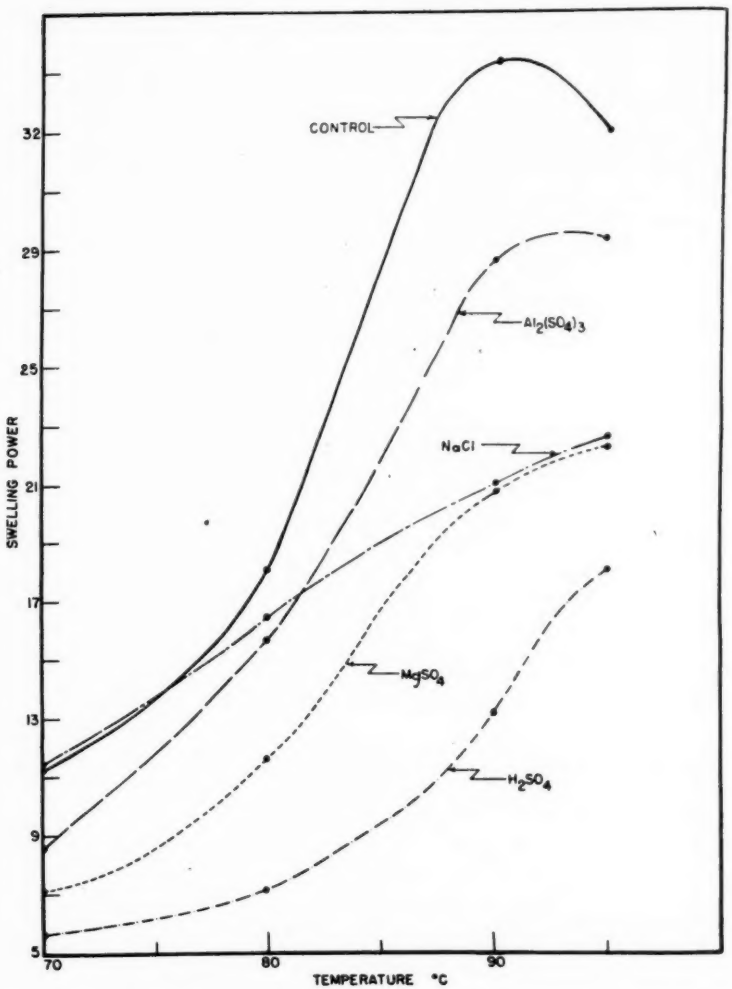

FIG. 5. Effect on swelling power of cooking starch, prepared by treatment with 0.125 N NaOH, in solutions containing cations of different valencies. Control was the same starch cooked in redistilled water.

cations which differ principally in valence. Of these, $MgSO_4$ has the greatest influence of the salts in reducing swelling power, particularly at lower cooking temperatures. At 90°C. $MgSO_4$ is approximately equal to NaCl in effect on swell. Below 80°C.(176°F.) the NaCl and control curves practically coincide and both are significantly above the others. Above 80°C. the swell, owing to $Al_2(SO_4)_3$, increases over that caused by NaCl but is definitely lower than the results obtained from cooking this starch with redistilled water, corresponding to the findings of Harris and Jesperson (1946). The influence of the addition of electrolyte at cooking to starch containing substituted cations was noted by Ripperton (1931) when working with root starch.

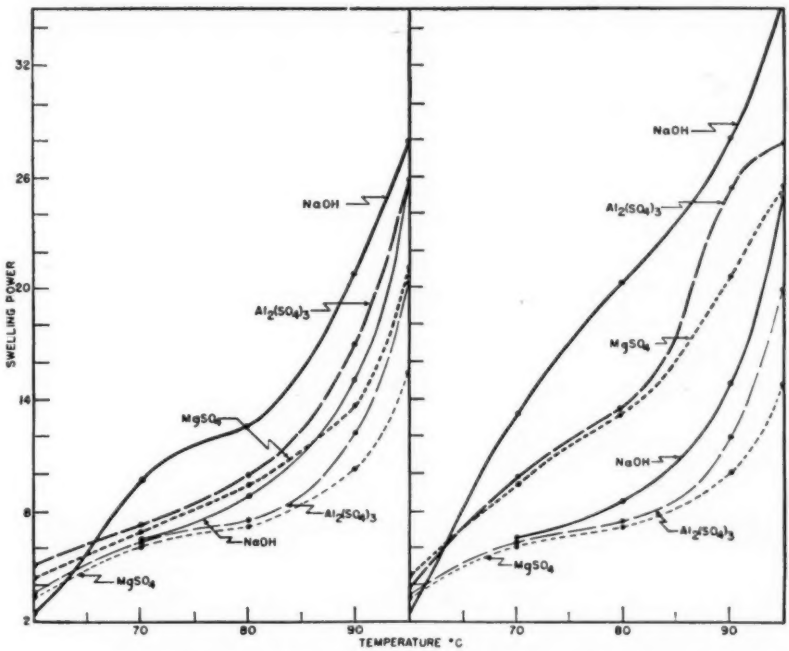

FIG. 6. Curves representing the effects of cooking two starches in solutions of NaOH, $MgSO_4$, and $Al_2(SO_4)_3$. Heavy lines represent wheat starch treated with chlorine, while light lines represent wheat starch prepared from flour-water dough. In the diagram on the left, the starch was treated with 0.007 N chlorine water, while on the right the treatment was 0.014 N chlorine.

Effect of Cooking Solution on Chlorinated Starch: The two chlorinated starches were cooked in solutions of NaOH, $MgSO_4$, and $Al_2(SO_4)_3$ and the resultant swell compared with corresponding cooking treatments made on wheat starch separated from flour doughs (Fig. 6). $MgSO_4$ decreased the swelling power most for both chlorinated starches, while $Al_2(SO_4)_3$ decreased it less but more than NaOH. The same order of cooking reagents is apparent in the swell of the starch prepared from flour dough by water

treatment, but the effect is much less marked because of the lower swelling power of the starch. The effect of the two salts corresponds to the results shown (Fig. 5) where $MgSO_4$ lowered the swell of starch prepared with NaOH much more than $Al_2(SO_4)_3$. The explanation of these effects are not apparent at the present time; no doubt there are a number of interlocking factors functioning to produce the final results.

The influence of various cooking solutions on starch prepared with sulphurous acid is shown (Table 2). Because of the very small differences in swell caused by the solutions until a relatively high temperature was attained, the data were not put in the form of a diagram. Sulphuric acid had the greatest effect in raising swell at 95°C., with $Al_2(SO_4)_3$ somewhat less. This is possibly due to the greater activity of the hydrogen ion at this temperature, while H_2SO_3 may tend to decompose at this point. Of the reagents, $MgSO_4$ gave the lowest swell at 95°C., but it is probable that the difference from the control was nonsignificant. The effect of H_2SO_4 in cooking starch prepared by use of H_2SO_3 corresponds to the results obtained on chlorine-treated starch.

TABLE 2

Effect of Cooking Solution on Swelling Power of Starch Prepared With Sulphurous Acid

Cooking solution	Swelling power at indicated temperature				
	60°C.	70°C.	80°C.	90°C.	95°C.
	gm./gm.	*gm./gm.*	*gm./gm.*	*gm./gm.*	*gm./gm.*
H_2SO_4	4.4	6.5	7.5	13.4	28.6
$Al_2(SO_4)_3$	3.3	6.5	7.7	14.9	24.9
Control (water)	4.2	6.8	7.9	12.0	18.4
$MgSO_4$	3.4	6.7	7.6	11.9	16.3

CONCLUSIONS

The complex situation involved in the effect of electrolytes in dilute concentration of starch gelatinization are implicit in the data presented in this report. There are no doubt many factors operative in affecting starch swell, and it is extremely difficult at this time to separate them and evaluate each according to effect. It is apparent that ions can so alter the physicochemical behavior of starch pastes that the original source of the starch has little bearing on the swell, provided that this source is a cereal, Harris and Jesperson (1946). Part of the explanation of the effect of electrolytes may lie in their effect on the secondary valence bonds active between different parts of the amylopectin constituent of starch. If these bonds are ruptured, the micellar structure is disorganized, larger units are formed, and the amylose molecules are now able to take up more water. A contributing factor may also be the effect of salts on water itself.

No intensive work on root starches has been done at this laboratory, and it is probable that the method employed would not be suitable for root starch because of the exceedingly high swell exhibited by this class of starch, Ripperton (1931). Root starch, when gelatinized, is markedly frail

and the granules break down and disintegrate very easily. Even with cereal starches discrepancy between replicates was greatest at 95°C.

SUMMARY

One commercial cornstarch and several other starches prepared from wheat flour in the laboratory were studied in respect to the effect of preparation and the electrolyte present during cooking on swelling power. It was found that chemical treatment during preparation of the starch had a marked effect upon starch properties. Treatment with 0.125 N NaOH at 5°C. raised the pH of the dry starch and greatly increased swell. Preparation with 0.123 N H_2SO_3 lowered pH and increased swell very slightly. Exposure of wheat starch to dilute solution of chlorine also lowered the pH and augmented the swelling power.

Cooking in the presence of acid greatly increased swelling at 90°C. and above. NaOH increased the swell even at 70°C.; the swell increased with temperature but was less at 95°C. than the swell in HCl. The valency of the cation appeared to affect swell; NaCl and $MgSO_4$ decreased it to some extent, while $Al_2(SO_4)_3$ and $Th(NO_3)_4$ increased swell above 90°C. Apparently H and OH ions were more effective in increasing swell than other ions studied.

Cooking starch prepared with NaOH in the presence of acid markedly decreased the swell. Cation valency influenced the results, decreasing the swell as compared with cooking the starch in water. The order of effect of cations on starch prepared with and without chlorine was similar, swell being less with $MgSO_4$ than with NaOH, while the effect of $Al_2(SO_4)_3$ was intermediate.

The study emphasizes the complexities involved in the problem of the influence of electrolytes on the swelling power of starch.

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PREPARATION OF CRUDE PLANT EXTRACTS AND THEIR ASSAY FOR PRESENCE OF ANTIBACTERIAL SUBSTANCES [1]

E. H. LUCAS, KATHRYN PEARSON, R. W. LEWIS, AND BETTY VINCENT

Michigan Agricultural Experiment Station, East Lansing, Michigan

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It has been found necessary to establish a method for testing crude extracts of plant materials, particularly of organs of higher plants, in order to organize the results of a wide survey made for the purpose of determining the occurrence of antibacterial substances. The initial phase of such an investigation is of necessity rather uncomplicated. It aims to discover the presence of substances with antibacterial properties in freshly prepared extracts. For the purposes of the survey it is immaterial whether these substances are present as such in the living plant tissues or whether they are formed in the process of maceration by interaction of originally inert chemicals. An aqueous extraction has always been the first approach. It is an inevitable step and will, in most cases, reveal whether the examined material yields antibacterial substances. Most of the substances extracted showed at least a slight solubility in water. In addition, as will be pointed out later, the amount of water used was in many instances so small that the question of water solubility could be neglected. However the possibility of a far better effect of other solvents must be considered. Hence routin extractions with ethanol, acetone, and chloroform were made as a rule.

That initial yields are of minor importance has been pointed out by Cavallito, Bailey, and Kirchner (1945). The crude extract does not indicate whether the antibacterial effect is due to the weak action of a substance present in appreciable quantities or to the action of minute amounts of a highly potent agent.

Although in the following description mainly aqueous extracts are being considered, extracts prepared with any other solvent may be tested in the same manner. In many cases it will be necessary to remove the solvent and take up the residue with water. However, neither ethyl ether nor acetone affect the growth of most of the organisms used in the test described; hence ether and acetone extracts can be used as such if this is desirable.

PREPARATION OF PLANT MATERIAL

The plant material to be tested may be macerated in a meat grinder or similar device and then expressed by means of a hydraulic press in order to obtain the undiluted crude juice. This procedure was found to be rather time-consuming. Besides, in cases of plant materials with little

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moisture, there is a certain possibility of overlooking antibacterial principles which might form by enzymatic hydrolysis in the presence of sufficient water. As the method of choice in exploratory tests the following procedure has been used in all cases in which the plant material contained a sufficient amount of moisture.

The material was cut into small pieces by means of a stainless-steel knife and disintegrated in a Waring blendor with an amount of distilled water, varying with the material used, but at any rate as small as possible. The water was employed as a mechanical aid in facilitating blending. Only to a limited extent was it expected to provide the extraction of water-soluble substances. Materials containing less moisture were blended with adequate amounts of water. In these cases the extraction of the water-soluble substances could be expected. More water was also used whenever a comparatively strong antibacterial activity was expected. It is in the nature of the bacteriological test which will be described here that small circles of inhibition should be obtained. Therefore, the dilution of any plant material should be carried to the point where small circles of inhibition (between 20 and 30 mm. diameter) are produced.

The quantities of plant material and water used must be in accordance with the size of the blender jar; for the standard size Waring blendor jar a minimum of 100 ml. of water is required; in the now available smaller jars proportionally smaller amounts may be used. After three to four minutes of blending the material was strained through four layers of cheesecloth or, in many instances, through muslin. Squeezing by hand was usually necessary to speed up the process of straining. In some experiments the extracts were filtered through paper with the aid of celite or similar materials. However, this may result in the loss of the antibacterial principles by adsorption on the filter aid. Most materials were centrifuged after straining. The supernatant liquid was decanted and thus ready for the assay. In all cases the residual material was taken up with water after centrifugation and tested separately.

ASSAY

For the assay of crude plant materials the method described by Vincent and Vincent (1944) was found superior to all other methods used. In general, the paper-disc method offers great technical advantages over the cup assay for the examination of crude plant materials. Besides, its accuracy has in all cases tested exceeded that of the various forms of assay methods in which glass, porcelain, or metal cylinders were used as carriers of the test material. A number of modifications of the Vincent test were necessary since the original assay was designed for the determination of the potency of penicillin. The modifications include the following:

1. The use of three test organisms. In addition to *Staphylococcus aureus*, the standard organism in penicillin assays, two gram-negative organisms, *Salmonella pullorum* and *Phytomonas phaseoli*, were chosen for quantitative work. It was logical to include a gram-negative organism in order to establish the presence of substances affecting them; *S. pullorum* was given preference over *Escherichia coli* which had been first used but was abandoned because of its great resistance and adaptability to adverse

conditions. The authors felt that its behavior did not represent that of the average of gram-negative organisms in which they were interested. *Phytomonas phaseoli* was chosen because of its great sensitivity to a wide range of antibacterial agents, the ease with which it can be carried and reproduced, and its nonpathogenicity toward humans and animals. Waksman, Bugie, and Reilly (1944) have emphasized that some plant pathogenic bacteria are more sensitive to certain agents and more resistant to others, while as a group the plant pathogens of the gram-negative type "are

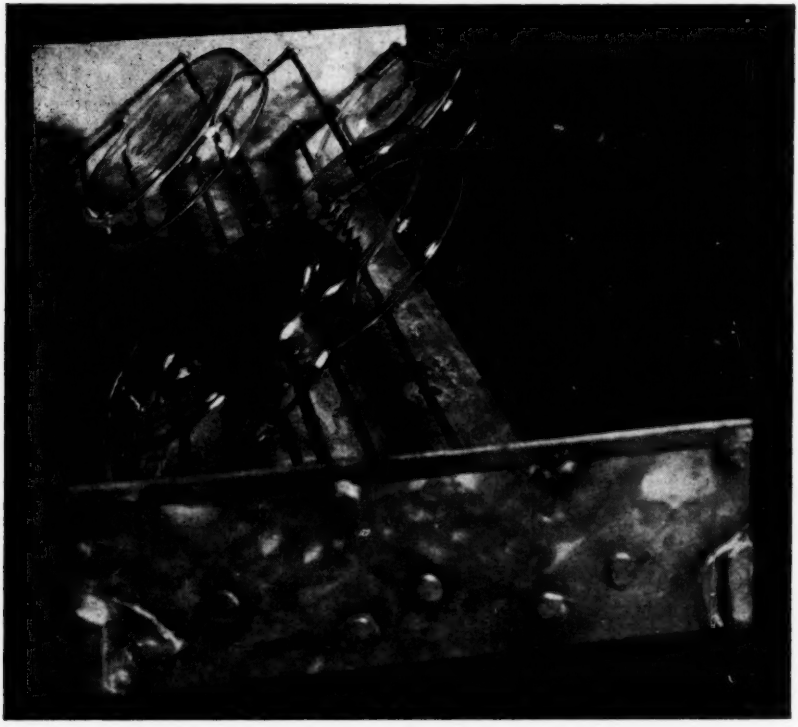

FIG. 1. Drying rack.

similar to the many animal pathogens of the same type, being more resistant than the gram-positive, and less resistant than the gram-negative bacteria of the Escherichia type."

The test organisms were cultured in tryptose broth and transferred every 24 hours. An 18 to 24-hour culture was used for inoculation of the agar.

2. Differences in the technique of preparing and inoculating the plates. In general, the technique recommended for the preparation of the plates was followed; however, they were not incubated after they had been poured. The surface moisture was removed by letting the plates stand at

room temperature for two hours. They were stored in a refrigerator at 4°C.(39.2°F.) for at least one hour and for not more than three days. Tryptose agar was used for *S. aureus* and *S. pullorum*, and nutrient agar (Difco) for *P. phaseoli*. Ten ml. of agar was used for each of the Petri dishes which had been specially selected for flat bottoms. One ml. of the culture broth was pipetted into each dish, and the excess was drained off by placing the inverted dish on a metal rack (Fig. 1) at an angle of 45 to 50 degrees.

3. Differences in handling the filter-paper discs. The filter-paper discs were sterilized by autoclaving and subsequently dried for 24 hours at 37°C.(98.6°F.). They were placed on the agar after having been immersed in the test fluid for five seconds. It is clear that immersion for an extended and even for a shorter period does not produce significantly different results (Table 1); the intermediate period was therefore chosen.

TABLE 1

Absorption of Water by Filter-Paper Discs[1] After Immersion for Different Periods

	Filter-paper discs immersed		
Amount of distilled water absorbed (milligrams)	1 sec.	5 sec.	10 sec.
	122.35±2.98[2]	123.44±3.67	122.21±2.33

[1] Schleicher and Schuell No. 740-E. [2] Standard deviation of mean of 15 determinations.

4. Evaluation. Readings were taken after an incubation of 18 hours at 37°C. for *S. aureus* and *S. pullorum* and at 27°C.(80.6°F.) for *P. phaseoli*. For the evaluation of the potency of antibacterial substances derived from plant materials a suitable standard was required. For this purpose infusions of dehydrated garlic[2] were used successfully. Ten grams of the powder was mixed with 125 ml. of distilled water in a small jar of a Waring blendor. The blender was operated for five minutes, the mixture strained through four layers of cheesecloth, and centrifuged at medium speed for five minutes. The supernatant liquid was filtered by suction through a No. 1 Whatman circle. Serial dilutions were made from this liquid to obtain one-half, one-fourth, one-eighth, and one-sixteenth of the original concentration. Intermediate dilutions were also made. By setting up octuplicates of each of these standard solutions (two discs per plate) it was found that a straight-line relation existed between the logarithm of the diameter of the zone of inhibition and the logarithm of the concentration of the infusion.

Since it was established that the points of the standard curve could be determined with at least 10 per cent accuracy the average curve obtained was set up as a reference curve for the evaluation of unknown plant extracts (Figs. 2, 3, 4). It is merely necessary, therefore to test one concentration of the garlic standard along with the unknown sample; if the reading of the standard obtained is within 10 per cent of the corresponding value on the reference curve, the potency of the unknown plant extract can be read on the curve.

[2] Obtained through courtesy of Basic Vegetable Products Co., Vacaville, California.

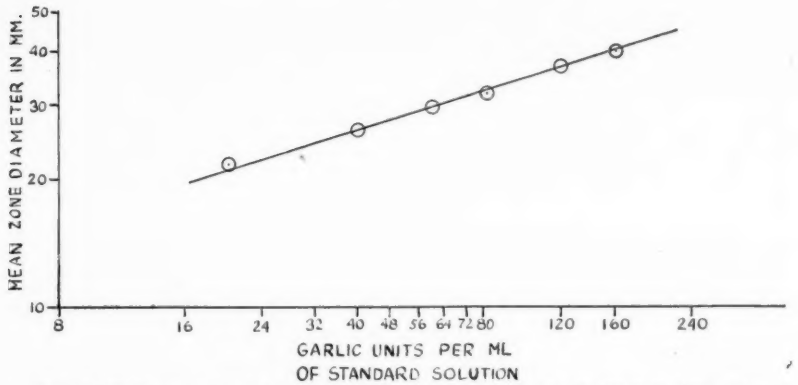

FIG. 2. Reference curve for *Staphylococcus aureus*, Strain H, M.S.C. (Department of Bacteriology).

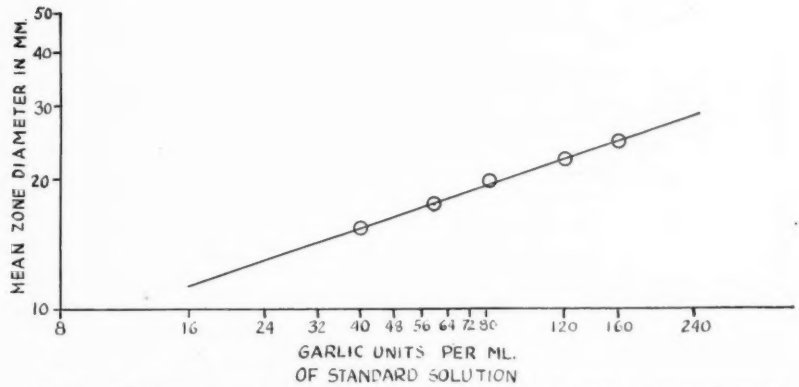

FIG. 3. Reference curve for *Salmonella pullorum*, Strain 3, M.S.C. (Department of Bacteriology).

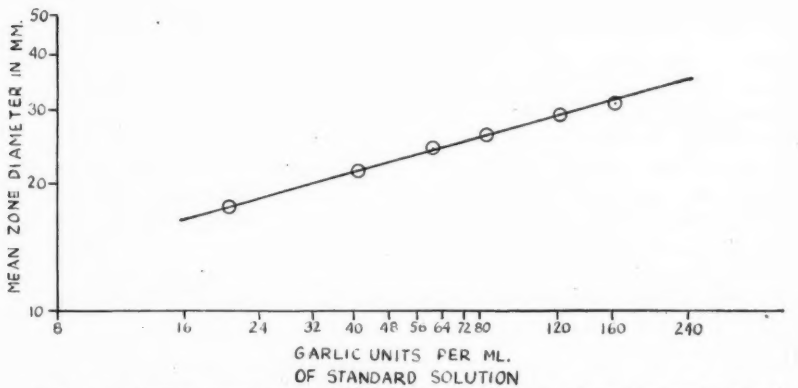

FIG. 4. Reference curve for *Phytomonas phaseoli*, M.S.C. (Department of Botany).

To facilitate the evaluation of unknown samples Table 2 was prepared. The standards were prepared freshly every time when plant materials were tested. For convenience the potency of the unknown extracts was expressed in garlic units. One garlic unit (g.u.) is the amount of active principle contained in one ml. of a solution prepared by blending 0.1 gm. of dehydrated garlic (Basic Vegetable Products Co.) with 400 ml. of distilled water in the manner described in this paper. Hence, the aforementioned concentrate (10 gm. in 125 ml.) had a potency of 320 g.u. and the dilutions had potencies of 160, 80, 40, and 20 g.u., respectively.

TABLE 2

Diameters of Circles and Potencies Obtained From Reference Curves

Water used per gram of garlic powder	Corresponding potency	Diameter of circle [1]		
		S. aureus [2]	*S. pullorum* [3]	*P. phaseoli* [4]
ml.	*g.u.*	*mm.*	*mm.*	*mm.*
200.0	20	21.9±44[5]	.....	17.6±0.99
100.0	40	26.4±0.73	15.9±0.74	21.1±1.51
66.6	60	29.8±0.93	18.3±0.73	23.6±0.92
50.0	80	32.1±0.55	20.4±0.81	25.3±1.42
33.3	120	37.1±0.78	23.2±0.73	28.3±0.89
25.0	160	40.4±0.75	25.4±0.71	30.0±1.38

[1] Means of 60-80 determinations for each concentration. [2] Strain H, M.S.C. Department of Bacteriology. [3] Strain 3, M.S.C. Department of Bacteriology. [4] M.S.C. Department of Botany. [5] Standard deviation of mean.

TABLE 3

Absorption of Liquids by Filter-Paper Discs [1] After Immersion for Five Seconds

Amount of liquid absorbed	Distilled water	Garlic powder, extract 1:50	Tomato fruits, extract 1:4	Lettuce leaves, extract 1:10
(milligrams).....	123.4±3.67[2]	125.3±3.45	122.9±4.37	124.8±3.70

[1] Schleicher and Schuell No. 740-E. [2] Standard deviation of mean of 25 determinations.

It was established that plant extracts were taken up by the filter-paper discs at a very uniform rate and that the average quantity absorbed was 0.124 ml. (Table 3). The authors are familiar with the method proposed by Loo, Skell, Thornberry, Ehrlich, McGuire, Savage, and Sylvester (1945), which makes use of the application of a measured amount of liquid to the paper disc. While they admit that different surface activity of the materials used may slightly alter the volume of the liquid absorbed, they feel that in the evaluation of crude materials approximations are entirely sufficient. It is equally important to realize that one standard is here employed for the characterization of principles which may vary widely in their physical properties. As Waksman, Bugie, and Reilly (1944) stated, no single standard can be used in evaluating different antibacterial substances since they vary in their rate of diffusion. With crude materials this precaution can be disregarded.

The dehydrated garlic has been obtained so far in a remarkably uniform quality. Although several batches have been used and some of them stored for as long as one year, the reference curve set up is still unchanged.

The active principle which has remained in the dehydrated material evidently is very stable. (There is no doubt that certain substances are being lost in the process of drying.) To avoid the necessity of corrections of the reference standard, a quantity of the dehydrated material originally used may be stored under conditions precluding its deterioration.

A standard solution of a given potency will cause a circle of inhibition of different diameters when it is set up against different organisms. For example, the diameter of a zone of inhibition corresponding to 80 g.u. is 32.1 mm. on a culture of the *S. aureus* strain used, 20.4 mm. on a *S. pullorum* culture, and 25.3 mm. on a *P. phaseoli* culture. It must be considered, therefore, in the evaluation of unknown substances that they are being compared with a principle that itself is more potent against gram-positive organisms than against gram-negative organisms, as shown by the action against *S. aureus* and *S. pullorum*. Moreover, the principle used as standard is also less active against *P. phaseoli* than against *S. aureus*. This is remarkable because *P. phaseoli*, as was pointed out before, represents a group of rather sensitive plant pathogens which ordinarily are more susceptible to the action of antibacterial compounds than gram-positive organisms. Similar observations had been published by Waksman, Bugie, and Reilly (1944).

There is a wide variation in the reaction of strains of microorganisms to chemical substances. Tainter, Miller, and Becker (1946) have recently reviewed certain aspects of this problem, particularly with reference to sulfonamide and penicillin fastness. It is of paramount importance to use in the tests the strains with which the original standard readings were established. It is necessary to set up standards with each of the unknown samples.

SUMMARY

A method of testing crude plant extracts for the presence of antibacterial substances is described. A stable standard of vegetable origin is introduced and its use discussed.

ACKNOWLEDGMENT

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EDITORIAL REVIEW

MUSTINESS IN FOODS

L. B. JENSEN
Research Laboratories, Swift & Company, Chicago 9, Illinois

Those engaged in food processing and storage may on occasion encounter an organoleptic entity of foodstuffs called "mustiness," "a moist loam odor," or "an earthy odor." In some instances the odor of the affected food is penetrating enough to permeate not only the food itself but the storage room as well. Many pure cultures of microorganisms isolated from these sources emit such penetrating odors that the microbiologist is forced to destroy his cultures and seal his stock strains. Mustiness may occur in eggs, fish, grains, potatoes, nut meats, milk, butter, cheese, beef, veal, lamb, pork, and poultry meats. The flora may be quite different in the several foods. Experience has shown that moist foods when showing mustiness also show the presence of *Pseudomonas* and *Achromobacter*. Some of the moist foods and most of the drier foods, when musty, may harbor actinomycetes of the genera *Actinomyces* and *Streptomyces*.

THE BACTERIA

Turner (1927) isolated a strong, musty-odor-producing organism from musty eggs which he named, *Achromobacter perolens*. The problem of musty or "hay" eggs is an old one, and a comparatively large literature on "hay" eggs developed before Turner's work. However, specific organisms were apparently not isolated and described in the literature available to us.

Spanswick (1930) studied the cause of mustiness in eggs and also isolated a rod-shaped organism, which she classified as an *Achromobacter*. Turner had noticed that his cultures, upon transfer, tended to lose their pungency, whereas Spanswick's culture has always retained a characteristic odor. Levine and Anderson (1932), in their work on the bacteriology of musty eggs, described two species of *Pseudomonas* producing a penetrating musty odor in all mediums. Our strains of these cultures have now lost their musty odor.

Fifteen strains of musty bacteria were isolated from six different samples of veal. All of these strains proved to be *Pseudomonas graveolens* (Levine and Anderson) and *Achromobacter perolens* (Spanswick); the criteria for classification are listed (Table 1).

Two strains of musty bacteria were isolated from musty beef. These organisms were identical with *Pseudomonas graveolens*. Spanswick's *Achromobacter* was also isolated from musty poultry meats and poultry skins.

Loss of pungency after four transfers of cultures of *Pseudomonas graveolens* strains on nutrient agar was the rule. With Spanswick's type of *Achromobacter perolens* over 15 transfers made weekly did not alter the strains in this respect. New species or variants of musty bacteria have not been encountered with criteria employed in this laboratory (Table 1).

TABLE 1

Differential Characteristics of Musty Bacteria[1]

Species	Motile	Growth at		Agar plate colonies	Gelatin liq.	Acid in milk	NO_3 to NO_2	Lactose (5 days)	Arabinose (5 days)	Rhamnose		Agar colonies—fluorescence[2]	Source
		37°C.	10°C.							5 days	20 days		
Achromobacter perolens (Turner)	+	—	+	Opaque	+	—	+	—[3]	+[3]	—	?	?	Eggs
Achromobacter perolens (Spanswick)[4]	+	—	+	Translucent	—	—	—	—	—	—	—	+	Eggs
Pseudomonas graveolens (Levine and Anderson)	+	+	+	Opaque, butyrous	—	+	—	+	—	—	+	—	Eggs
Pseudomonas mucidolens (Levine and Anderson)	+	+	+	Opaque, butyrous	+	—	+	—	+	—	Alk.	—	Eggs
Pseudomonas mucidolens, var. tarda (Levine and Anderson)	+	+	+	Opaque, butyrous	+	—	+	—	+	—	+	—	Eggs
P-1 *Achromobacter perolens*	+	—	+	Translucent	—	—	—	—	—	—	—	+	Poultry skins
V-1 *Achromobacter perolens*	+	—	+	Translucent	—	—	—	—	—	—	—	+	Veal rolls
B-1 *Pseudomonas graveolens*	+	—	+	Opaque, butyrous	—	+	—	+	—	—	+	—	Beef and veal
L-1 *Pseudomonas graveolens*	+	—	+	Translucent	—	+	—	+	—	—	+	+	Pork loins
P-2 *Achromobacter perolens*	+	—	+	Translucent	—	—	—	—	—	—	—	+	Poultry meats

[1] All gram-negative, nonsporing, aerobic rods; all produce H_2S indol negative; incubation at 20°C.(68°F.). [2] Georgia and Poe (1931). [3] + = acid; — = no action. [4] American type culture collection (Spanswick) No. 4430.

The thermal death times of all of these strains are less than 62.8°C. (145°F.) for 30 minutes.

ACTINOMYCETES

Most of these common soil organisms, when grown on laboratory mediums, produce very penetrating, musty-earthy odors—Skinner, Emmons, and Tsuchiya (1947). These writers present an excellent chapter on the characteristics of actinomycetes. There are four genera: *Actinomyces, Nocardia, Streptomyces,* and *Micromonospora.* Most investigators agree that these organisms are difficult to classify. Practically any inocula of cultivated soils into agar mediums show, upon incubation, numerous species of the actinomycetes group present in large numbers. It is not surprising that these forms are encountered in foodstuffs.

Fellers (1922) studied many strains of actinomycetes found in musty milk. The earthy-musty-producing species commonly observed were *Actinomyces griseus, Actinomyces albus, Actinomyces rutgersensis, Actinomyces bobili,* and *Actinomyces lipmani.* Fellers found hay and straw harbored these organisms. He also observed mustiness of *Actinomyces* origin in walnut meats, dried-fish flakes, grains, and probably in some instances in eggs. These musty strains in both moist and dry places grew at low temperatures but not at 37°C.(98.6°F.). Refrigeration delayed mustiness in milk but did not prevent spoilage from this cause.

Thaysen (1936) discovered fish living in a tidal English river, the water of which was heavily contaminated with *Actinomyces,* acquired a musty or earthy taste. When the fish were seined from the river and kept in clean water for five days their flesh lost the earthy-musty flavor.

The characteristic odors of must or earth associated with dank cellars, wet haystacks, potato bins, freshly plowed soil, and wet piles of old sawdust usually originate from actinomycetes. A small growth of some of these forms in a storage bay can permeate the entire room with its offensive odor. *Actinomyces scabies,* the causative organism of potato scab, is described as associated with odors characteristic of these potatoes. Numerous examinations of musty meats, both fresh and cured, have failed to disclose the presence of actinomycetes in numbers suggesting growth. Butter, cream, cheeses, and other dairy foods have on occasion shown the effects of growth of actinomycetes, Richie (1927).

The thermal resistance of actinomycetes is usually less than 78°C. (172.4°F.) for 20 minutes. Their temperature range for growth is considered to be from 0 to 38°C. (32 to 100.4°F.), although thermophilic actinomycetes are not uncommon. We employ 20°C.(68°F.) for routine incubation, although 15 to 32°C.(59 to 89.6°F.) have been advocated as optimum temperatures. They do not grow well in acid mediums. We have never observed molds as the causative agents of true mustiness in foods.

ODORS FORMED BY MICROORGANISMS

Certain microorganisms may produce their characteristic odors other than mustiness when growing in foodstuffs. Claydon and Hammer (1939) observed that *Pseudomonas mephitica* produced a skunk-like odor in butter. This organism grows at 21°C.(69.8°F.), but not at 37°C.(98.6°F.).

On nutrient agar the colonies are shiny gray with wrinkled centers and doughy consistency. Gelatin is liquefied; indol and hydrogen sulfide are not formed; fats are not hydrolyzed; nitrates are reduced to nitrites; and ammonia is formed. In milk, meat, and protein mediums a skunk-like odor is noticeable. *Flavobacterium proteus* produces a parsnip-like odor in milk, meat, and protein mediums. This organism grows best at 18°C. (64.4°F.); does not liquefy gelatin; reduces nitrates to nitrites and does not coagulate, peptonize, or acidify milk. The colonies on nutrient agar are yellow. Glage's (1901) *Pseudomonas* is commonly found on beef stored at 3.3°C.(38°F.) for maturation (tenderness and flavor) and imparts a desirable black walnut-like flavor to the meat. Omelianski (1923), in a review of specific odors both pleasant and unpleasant resulting from growth of microorganisms on laboratory mediums, milk, fruits, and carbohydrate solutions, described a variety of aroma-producing bacteria, yeasts, and molds. He suggested that some odors formed by microorganisms, such as those from strawberries, pineapple, apple, pear, bitter almond, muskmelon, and turnips, depend upon the medium used. Other microorganisms produced their characteristic aroma on suitable laboratory mediums. The odor of certain petroleum compounds is on rare occasions perceived in beef. Coale (1947) discusses the drinking of crude oil by cattle pastured in oil fields, which imparts a characteristic organoleptic defect to the entire beef musculature.

DISCUSSION

There is no information available to us on the chemical nature of the musty compounds formed by microorganisms. Moncrieff (1946) describes the odor of diphosgene as a "musty hay smell." There is very little likelihood that carbonyl chloride ($COCl_2$) is formed by *Pseudomonas, Achromobacter, or Actinomyces.* Broth cultures of these musty microorganisms are not toxic to laboratory animals, nor are musty eggs and musty meats toxic to man. Boiled and fried meats (pork loins and veal) showing strong mustiness *(Achromobacter perolens* and *Pseudomonas graveolens)* were eaten by two men in 225-gram quantities without ill effects. Culinary treatments did not efface the organoleptic defect of mustiness. Cultures of *Pseudomonas graveolens* which had lost their musty properties produced strong musty odors on nutrient agar after growth on fresh pork. Nutrient agar cultures of the inoculated pork were faintly musty and, upon one transfer on nutrient agar, were again non-musty although a faint fruity odor was perceptible.

Spanswick's strain of *Achromobacter perolens,* which has never lost its characteristics, has been used in this laboratory as a test organism for determining antiseptic and germicidal properties of chemical compounds in various substances, such as liquid glue and other mediums. Since growth of this organism is accompanied with marked mustiness of the treated mediums, other laboratory examinations are not needed in presumptive tests for inhibitory properties. Slight growth suffices to bring out perceivable mustiness.

Control of musty microorganisms in food plants may be accomplished by employing the methods described elsewhere, Jensen (1945).

SUMMARY

Mustiness in foods is caused by the growth of *Pseudomonas, Achromobacter,* and *Actinomyces.* Foods commonly affected are eggs, fish, veal, lamb, pork, grains, potatoes, nut meats, milk, butter, cheese, and poultry meats. Moist foods, when showing mustiness, show the presence of *Achromobacter perolens* or *Pseudomonas graveolens.* Many strains of musty *Pseudomonas* lose this characteristic upon repeated subcultures on nutrient agar. Strains other than *Achromobacter perolens* and *Pseudomonas graveolens* have not been encountered in musty meats. Differential characteristics for identification of these bacteria are described. *Achromobacter perolens* may be useful as a test organism for presumptive determination of antiseptic and germicidal properties of chemical compounds since slight growth is accompanied with perceptible mustiness of the treated medium.

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THIAMIN CONTENT OF TROPICAL FOODS[1]

CONRADO F. ASENJO

Department of Chemistry, School of Tropical Medicine of the University of Puerto Rico, under the auspices of Columbia University, San Juan, Puerto Rico

With the co-operation of

ORO DE BORINQUEN SEGUNDO AND HILDA GARCÍA DE LA NOCEDA

Agricultural Experiment Station, University of Puerto Rico, Río Piedras, Puerto Rico

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There is little available information in the literature relative to the thiamin content of tropical foods. It is the purpose of the present paper, therefore, to report the results of an investigation undertaken to determine the thiamin content of cereals, fruits, vegetables, and other miscellaneous foodstuffs either produced or consumed in Puerto Rico and neighboring islands.

The average Puerto Rican basic diet is principally composed of rice, beans, root crops, dried codfish, plantain, and a small amount of meat. Among the low-income group of the population—by far the largest—dairy products are almost totally absent from the diet. On the basis of the data reported herewith, this basic diet alone, without meat or dairy products, supplies about 900 μg. of thiamin per 2,500 calories, which value is not far below the allowance of 1,200 μg. per 2,500 calories recommended by the Nutrition Board of the National Research Council (1945). As it is well-known that National Research Council standards are purposely intended to provide a wide margin of safety against individual variations in foods, the deficit in the Puerto Rican basic diet should not be considered dangerously low. The bulk of the thiamin in the average diet is contributed by the dried beans and root crops, which together account for more than 25 per cent of the weight of the whole diet.

Although border-line cases of polyneuritis are not uncommon, it is interesting to note that typical beriberi is practically unknown in Puerto Rico. In 1918, Gutiérrez [quoted by Ashford (1922)] observed several cases of "some form of beriberi" among the draft troops at Camp Las Casas, Santurce, P. R. Four years later, Ashford (1922) reported 12 cases of a similar syndrome in the military garrison at San Juan. These are, as far as the author can find out, the only cases of a beriberi-like syndrome so far reported in the local medical literature.

MATERIAL AND METHODS

All the foods assayed were purchased at various local markets or from street vendors in the San Juan-Río Piedras area and are, therefore, rep-

[1] Published with approval of the director of the School of Tropical Medicine and the director of the Agricultural Experiment Station of the University of Puerto Rico. A co-operative project.

resentative of what the average consumer buys; in the majority of cases five different samples of each material were assayed. The number of samples assayed, as well as the maximum and minimum values obtained and the average thiamin content for the particular material, are recorded (Table 1).

With but minor modifications the assay procedure followed was that of Conner and Straub (1941). The instrument employed was a Coleman Model 12 electronic photofluorometer. Clarase was used as the hydrolyzing enzyme.

The vegetables have been tabulated with the scientific nomenclature accorded to them by Britton and Wilson (1932). The common English names used are those recorded by the last-named authors or by Otero, Toro, and Otero (1945).

SUMMARY

The thiamin content of 87 articles of food important in the diet of Puerto Rico and neighboring islands was determined by the thiochrome method.

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TABLE 1

Thiamin Content of Tropical Foods

Material	Common name in Spanish	Description of sample	Number of samples tested	Thiamin in fresh edible portion		
				Max.	Min.	Av.
				μg./ 100 gm.	*μg./ 100 gm.*	*μg./ 100 gm.*
Vegetable products:						
Cereals and cereal products:						
Corn (*Zea mays* L.)	Maiz	Fresh from the cob	5	203	84	126
Rice (polished) (*Oryza sativa* L.)	Arroz pulido	Imported	4	130	54	88
Rice (whole) (*Oryza sativa* L.)	Arroz	Imported	2	310	300	305
Flour:						
Plantain flour (*Musa paradisiaca* L.)	Harina de plátano verde	Green plantain dried in oven and finely ground	1	.....	.....	176
Fruits:						
Avocado (*Persea Persea* L.)	Aguacate	Ripe	5	88	40	57
Banana, var. gigante (*Musa sapientum* L.)	Guineo, var. gigante	Ripe	4	51	20	35
Banana, var. morado (*Musa sapientum L.*)	Guineo, var. morado	Ripe	4	60	43	48
Banana, fig. (*Musa sapientum* L.)	Guineo, niño	Ripe	4	58	25	34
Breadfruit (*Artocarpus communis* Forst)	Panapén	Ripe	5	111	77	99
Cashew nut fruit (*Anacardium occidentale* L.)	Pajuil	Ripe; pulp	1	.....	.....	15
Cayure	Cayure	Ripe	5	60	28	39
Coco plum (*Chrysobalanus icaco* L.)	Hicaco	Ripe	4	16	8	12
Custard apple (*Annona reticulata* L.)	Corazón	Ripe	5	73	50	63
Genipa (*Genipa americana* L.)	Jagua	Ripe	3	31	18	24
Grapefruit (*Citrus maxima* Burm)	Toronja	Juice	6	32	15	23
Grapefruit (*Citrus maxima* Burm)	Toronja	Whole edible part	6	60	35	47
Guava (*Psidium guajava* L.)	Guayaba	Wild variety ripe	5	80	38	59
Kanapy, bullace plum (*Melicocca bijuga* L.)	Quenepa	Ripe	6	21	5	11
Lemon, native (*Citrus limonia* Osbeck)	Limón	Juice of ripe fruit	6	20	4	12
Mamey (*Achras sapota* L.)	Mamey zapote	Ripe	6	20	13	16
Mango (*Mangifera indica* L.)	Mango	Ripe; Mayagüez variety	6	50	25	35
Mesple (*Sapota achras* Mill)	Níspero	Ripe	5	6	1	2
Orange, sour (*Citrus aurantium* L.)	Naranja	Ripe; pulp of fruit	1	.....	.....	92
Orange, sour (*Citrus aurantium* L.)	Jugo de naranja	Juice of ripe fruit	5	57	17	36
Orange (*Citrus sinensis* L.)	China	Juice of ripe fruit	6	104	38	56

TABLE 1 *(Continued)*

Material	Common name in Spanish	Description of sample	Number of samples tested	Thiamin in fresh edible portion		
				Max.	Min.	Av.
				μg./ 100 gm.	*μg./ 100 gm.*	*μg./ 100 gm.*
Orange, native (*Citrus sinensis* L.)	China	Ripe; pulp and juice	6	125	62	86
Papaw (*Carica papaya* L.)	Papaya o lechoza	Ripe	6	14	6	10
"Pepino angolo" (*Sicana odorifera* Vell.)	Pepino angolo	Ripe	5	136	52	78
Pineapple (*Ananas Ananas* L.)	Piña	Ripe (whole)	5	98	55	74
Pineapple (*Ananas Ananas* L.)	Piña	Juice of ripe fruit	5	42	18	29
Pinguin, Maya fruit (*Bromelia pinguin* L.)	Maya	Juice of ripe fruit	6	83	71	80
Pomegranate (*Punica granatum* L.)	Granada	Ripe	5	32	20	25
Soursop (*Annona muricata* L.)	Guanábana	Ripe	6	71	35	57
Spanish plum (*Spondias purpurea* L.)	Jobillo	Ripe	2	55	48	52
Star apple (*Chrysophillum cainito* L.)	Caimito	Ripe	6	69	23	41
Strawberries (native) (*Rubus rosaefolius* Smith)	Fresas	Ripe	5	20	12	15
Tamarind (*Tamarindus indica* L.)	Tamarindo	Pulp surrounding seeds; ripe	6	590	300	370
West Indian cherry (*Malpighia punicifolia* L.)	Acerola	Ripe; pulp and skin	6	33	12	24
West Indian locust (*Hymenea courbaril* L.)	Algarrobo	Yellow powder surrounding seeds; ripe	6	126	40	82
Vegetables:						
Root crops:						
Arracacha (*Arracacia zanthorrhiza* Bancroft)	Apio	As sold in the market	5	90	47	65
Carrot (*Daucus carota* L.)	Zanahoria	As sold in the market	5	39	33	37
Cassava (*Manihot Manihot* L.)	Yuca	As sold in the market	5	108	86	92
Corn root, sweet (*Calathea allouia* Aubl.)	Lerenes	As sold in the market	3	41	22	29
Marunguey (*Zamia portoricensis* Urban)	Marunguey	Fresh	2	22	9	16
Radish (*Raphanus sativus* L.)	Rábano	As sold in the market	5	35	16	23
Sweet potato (*Ipomoea batatas* L.)	Batata	As sold in the market	6	130	65	89
Yam, white (*Dioscorea alata* L.)	Ñame blanco	As sold in the market	4	123	40	77
Yautía, white (*Xanthosoma sagittifolium* L.)	Yautía blanca	As sold in the market	9	195	95	130
Yautía, yellow (*Xanthosoma atrovirens* C.)	Yautía amarilla	As sold in the market	4	230	111	161

TABLE 1 *(Continued)*

Material	Common name in Spanish	Description of sample	Number of samples tested	Thiamin in fresh edible portion		
				Max.	Min.	Av.
				μg./ 100 gm.	*μg./ 100 gm.*	*μg./ 100 gm.*
Beans:						
Black beans (*Dolichos lablab* L.)	Chícharos	Dried; native	6	840	660	730
Cowpea (*Vigna unguiculata* L.)	Frijoles	Dried; native	6	1,100	700	847
Navy beans (*Phaseolus vulgaris L.*)	Habichuelas blancas	Dried; imported	5	750	360	585
Pigeon pea (*Cajanus cajan* L.)	Gandules	Partly dried; native	7	386	220	306
Red kidney beans (*Phaseolus vulgaris* L.)	Habichuelas coloradas	Dried; imported	5	640	460	534
Red kidney beans, native (*Phaseolus vulgaris* L.)	Habichuelas coloradas nativas	Dried; native	6	640	400	508
Rose beans (*Phaseolus vulgaris* L.)	Habichuelas rositas	Dried; imported	5	900	260	649
String beans (*Phaseolus vulgaris* L.)	Habichuelas tiernas	Fresh; native	6	109	42	75
Greens:						
Lettuce (*Lactuca sativa* L.)	Lechuga	Fresh leaves	5	60	39	46
Cucumber (*Cucumis sativus* L.)	Pepino	Fresh	3	37	23	29
Okra (*Abelmoschus esculentus* L.)	Quimbombó	Fresh	5	96	32	62
Peppers, green (*Capsicum frutescens* L.)	Pimiento verde	Fresh	5	45	25	36
Peppers, sweet (*Capsicum frutescens* L.)	Ajíes dulces	Fresh	5	109	56	80
Water cress (*Roripa nasturtium aquaticum* L.)	Berros	Fresh	3	55	41	42
Seeds:						
Almond, tropical (*Terminalia catappa* L.)	Almendra tropical	Kernel	4	473	369	422
Breadfruit, seed (*Artocarpus integrifolia* L.)	Pepita de pan	Edible part	6	258	64	180
Cashew nuts (*Anacardium occidentale* L.)	Castañas de Pajuil	Attached to fresh fruit	3	680	466	601
Coconut (*Cocos nucifera* L.)	Coco seco	Flesh from dried coconut	4	82	14	38
Coconut (*Cocos nucifera* L.)	Coco seco	Water from dried coconut	4	5	2	3
Other types:						
Calabash (*Crescentia cujete* L.)	Higüera	Ripe	2	44	40	42
"Calabaza de Castilla" (*Fevillea cardifolia* L.)	Calabaza de Castilla	Ripe	3	25	18	22
Chayote (*Sechium edule* Jacq.)	Chayote	Ripe	6	26	14	22
Eggplant (*Solanum melongena* L.)	Berenjena	Ripe	6	62	40	54
Onion (*Allium cepa* L.)	Cebolla	Whole	5	31	18	22
Plantain, green (*Musa paradisiaca* L.)	Plátano verde	As sold in the market	7	112	52	82

TABLE 1 *(Concluded)*

Material	Common name in Spanish	Description of sample	Number of samples tested	Thiamin in fresh edible portion		
				Max.	Min.	Av.
				μg./ 100 gm.	*μg./ 100 gm.*	*μg./ 100 gm.*
Squash (*Cucurbita moschata* Duch)	Calabaza	Ripe	7	96	48	73
Tomato (*Lycopersicon Lycopersicon* L.)	Tomate	Ripe	5	48	33	41
Animal products:						
Dairy products:						
Cheese (*Puertorican*)	Queso puerto-rriqueño	Resembles cottage cheese	4	30	9	21
Milk (cow's)	Leche de vaca	Pasteurized (bottled)	2	29	27	28
		Raw (retail)	2	17	17	17
Eggs:						
Hen's egg (yolk and white) together	Huevo de gallina (yema y clara) juntas	Purchased at random in different markets	6	164	40	77
Fish:						
Dried salted codfish	Bacalao	Imported from Nova Scotia	4	100	65	84
Meat:						
Rabbit meat	Carne de conejo		3	71	51	61
Miscellaneous:						
Casabe bread	Casabe	A crispy cracker made of meal prepared from cassava	1	.....	.....	62
Ginger (*Zingiber Zingiber* L.)	Gengibre	As sold in the market	4	29	15	21
"Guarapo" (*Saccharum officinarum* L.)	Guarapo	Fresh juice from cane	2	25	21	23
Honey (bee's)	Miel de abeja	Local product	5	20	4	7
Molasses, edible	Melao	Prepared by concentrating cane sugar juice	5	90	45	59
Yeast (*Torula utilis*)	Levadura	Manufactured at the pilot plant of "Cía de Fomento de P.R."	3	2,864	2,600	2,693

AN IMPROVED STONE MEDIUM FOR THE ISOLATION AND TESTING OF FOOD-POISONING STAPHYLOCOCCI

GEORGE H. CHAPMAN

Clinical Research Laboratory, 604 Fifth Avenue, New York 20, New York

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Rapid bacteriological study of food-poisoning outbreaks is essential for the prevention of recurrences. Direct application of the coagulase test, Chapman (1944a), to suspected foods and handlers has proved useful for rapid presumptive field investigation, Chapman and Domingo (1946), but the results must be confirmed by cultural methods. The *in vivo* demonstration of gastroenterotoxigenicity is desirable to add weight to the scientific evidence but it does not lend itself to routine testing of the numerous complaints of "food poisoning" received by health authorities and, because so many strains not associated with outbreaks also produce gastroenterotoxin, the results are not as conclusive as is generally thought [e.g., Fulton (1943)]. For epidemiological purposes, therefore, a rapid, simple, highly reliable bacteriological procedure would be of inestimable value to the busy sanitarian. The development of such a method will be described here. No claim is made for identification of food-poisoning staphylococci "with certitude," indeed absolute accuracy is a biological rarity.

Stone (1935) proposed a culture-medium technic by which food-poisoning staphylococci give a clear zone of gelatinolysis around the colonies on a special gelatin-agar medium. As originally applied the method proved to be nonspecific because many strains from outbreaks gave a negative reaction and many pathogenic and nonpathogenic strains from other sources produced considerable zoning. Later, Stone (1938) maintained that, if the test is applied promptly after isolation, nearly all implicated staphylococci show this zoning. Chapman, Lieb, and Curcio (1937) pointed out that the reaction is applicable only to pigmented strains. They found that, in addition to the Stone reaction, typical food-poisoning staphylococci produce pigment, coagulate plasma, and ferment mannitol and have other properties that are characteristic of pathogenic staphylococci. Other investigators claimed that many food-poisoning staphylococci do not give these reactions but a survey of the literature, Chapman (1942), showed that about 95 per cent of the published results of these tests were positive. Most of the organisms that were reported as negative were shown either to have had no established relation to the outbreak or to have been tested by methods other than those specified. The more recent literature shows a similar high proportion of positive reactions to these tests, Chapman (1947a).

In a series of studies in which the Stone reaction was applied directly to an improved isolation medium [Chapman (1946a) and Chapman and Domingo (1946)] all strains incriminated in local outbreaks showed considerable zoning in addition to producing pigment, coagulating blood, and

fermenting mannitol. This method proved to be suitable for routine testing of suspected staphylococcus food-poisoning outbreaks. By its use food-poisoning staphylococci were detected in large numbers in the incriminated foods, whereas another laboratory found none by "standard" methods. Bacto phenol red mannitol agar with 70 grams of sodium chloride added per liter, Chapman (1945a), was used parallel with the new medium because of its value when pathogenic staphylococci are heavily outnumbered by other bacteria, particularly by nonpathogenic staphylococci. To study other aspects of the outbreaks the samples were also plated on a new selective medium for coliforms, Chapman (1947c), and the selective differential streptococcus medium previously described, Chapman (1944b, 1946b, 1947e).

The present paper concerns itself with the development of a still further improved and even more simple technic for studying suspected staphylococcus food-poisoning outbreaks. A larger series of outbreaks is under study. If the results should be confirmed by others the "standard methods" isolation medium (blood agar) should be replaced by this medium because contaminants which are eliminated by the Chapman-Stone medium frequently overgrow staphylococci on blood agar, causing the negative results often obtained, and because blood-agar growths do not indicate with any degree of precision which colonies are likely to be those of food-poisoning staphylococci.

The culture medium (Chapman-Stone medium), which may be used both for selective isolation and for testing different properties of staphylococci, was developed as a result of the following observations. Sodium chloride provides selective isolation of staphylococci, improves chromogenesis, and is essential for the coagulation of blood, Chapman (1945a). Mannitol enhances chromogenesis far better than does lactose and, because carefully controlled fermentation of mannitol is a useful adjunct indicator of probable pathogenicity of staphylococci, Chapman, Lieb, and Curcio (1938), it serves a double purpose. Lactose, in a concentration of 0.2 per cent, increases the luxuriance of growth of staphylococci and does not interfere with tests of fermentation of mannitol. Bacto yeast extract is decidedly more favorable for chromogenesis than is meat extract. In addition to its use for the Stone reaction, gelatin increases the chromogenic property of staphylococci. Not all peptones are suitable for chromogenesis, and Bacto tryptone (but not tryptose) gave about the best results. Dibasic potassium phosphate is used to control the reaction which is of paramount importance not only for luxuriance of growth but also for the development of the chromogenic and coagulating principles. When the ingredients are mixed in optimum proportions and agar is added, the medium (staphylococcus medium No. 110) not only gives maximum selectivity and sensitivity in the isolation of all types of staphylococci but it also gives excellent development of the chromogenic property and serves admirably for the coagulation of plasma and blood, for the detection of acid produced from mannitol, and for the Stone reaction. Conditions that favor chromogenesis are favorable for the other reactions also. The medium has the following composition:

Water	1,000	ml.
d-Mannitol (Difco)	10	grams
Bacto tryptone	10	grams
Bacto agar	15	grams
Sodium chloride, Merck reagent	75	grams
Bacto-lactose	2	grams
Bacto yeast extract	2.5	grams
K_2HPO_4, anhydrous, Merck reagent	5	grams
Bacto-gelatin	30	grams

If necessary, adjust the reaction to pH 7.0. Do not substitute any of the ingredients without first comparing the resulting medium with the one made from the brands specified or with the Difco dehydrated medium, experimental lots of which have given satisfactory results.

Because most foods contaminated with food-poisoning staphylococci develop the toxic principle in a few hours in a warm environment [about 30°C.(86°F.)], a number of cultures were incubated on staphylococcus medium No. 110 at 30 to 31°C.(86 to 87.8°F.). Many of them produced deeper pigment and clotted blood faster than they did at 37°C.(98.6°F.), Chapman (1947b). Mannitol fermentation and the Stone reaction were above as active at 30°C. as they were at 37°C. Therefore, it is recommended that cultures on staphylococcus medium No. 110 be incubated at 30°C. A longer interval (48 hours instead of 42 to 43 hours) is necessary because of the slightly lower metabolic activities at the lower temperature.

The Stone reaction was applied originally by flooding the incubated plates with a saturated solution of ammonium sulfate. However, it is difficult to dispose of the plates without spilling contaminated liquid and the presence of ammonium sulfate solution interferes with isolation of pure cultures and makes it impossible to save the plates for future study. Ammonium sulfate can be added to staphylococcus medium No. 110 before sterilizing it, the optimum concentration being 75 grams per liter. An A.C.S. reagent grade is essential. Baker's pyridine-free ammonium sulfate "suitable for blood analysis" gave best results. The acidity of this chemical must be neutralized. Best results are obtained by adding 6.0 ml. of 10-per cent sodium hydroxide for each 75 grams. This liberates ammonia but it does not interfere with growth of staphylococci. The medium need only be sterilized for 10 minutes at 15 pounds pressure. On such a medium the colonies of staphylococci are considerably smaller than they are on staphylococcus medium No. 110, but this fault is largely eliminated by omitting the lactose and reducing the sodium chloride to 55 grams per liter. This modified medium (Chapman-Stone medium) has the following composition:

Water	1,000	ml.
d-Mannitol (Difco)	10	grams
Bacto tryptone	10	grams
Bacto-agar	15	grams
Sodium chloride, Merck reagent	55	grams
Bacto yeast extract	2.5	grams
K_2HPO_4, anhydrous, Merck reagent	5	grams
Bacto-gelatin	30	grams
Ammonium sulfate, Baker analyzed, A.C.S. pyridine-free	75	grams
Sodium hydroxide, 10 per cent	6	ml.

Sterilize 10 minutes at 15 pounds pressure. While still hot, shake thoroughly to disperse the precipitate and pour 40 plates from each liter. If the plates are poured when the medium is too cool, it will be lumpy and there will be pitting from air bubbles. Do not let the flask stand on a cool surface while it is cooling or the adjacent medium will solidify, making the medium lumpy. When properly prepared, the medium is uniformly opaque and free from coarse particles, lumps, and pits. It is translucent when warm but becomes opaque on cooling to about 25 to 30° C. (77 to 86° F.).

When cultures on this Chapman-Stone medium are incubated for 48 hours at 30 to 31°C., they develop deeper pigment and coagulate blood far more rapidly than they do by any previous method, including the staphylococcus medium No. 110. Acid production from mannitol is detected by adding a drop of 0.04-per cent bromcresol purple to the cleared area left after scraping the growth for the coagulase test. Any change in the color, compared with that on an unused area of the medium, indicates that mannitol has been fermented. The Stone reaction, a clear halo around the growth, is as intense as it is on staphylococcus medium No. 110, even though the growth is not quite as luxuriant as it is on the latter medium. Because of the uniform conditions under which the Stone reaction is produced, the width of the zone is remarkably constant. Therefore, instead of reporting the reaction as +, ++, etc., the total diameter of the clearing can be measured in millimeters.

The medium is excellent for study of all types of staphylococci, including nonpathogenic and G forms. However, it is unsuitable for keeping stock cultures because ammonium sulfate crystallizes when moisture evaporates from the surface of the medium, making staphylococcus medium No. 110 more desirable for this purpose. When protected from surface dehydration the cultures will remain viable and undergo no loss of coagulating power when stored in the refrigerator for a month or more. Both uninoculated plates and those containing growths can be stored in the refrigerator by the method of Chapman (1947d), in which they are kept in cans sealed with waterproof adhesive tape.

The following methods are used to study staphylococcus growths that occur on the Chapman-Stone medium. Any pigmented colony surrounded by a clear halo is likely to be a food-poisoning staphylococcus. If the growth is too scanty, transfer it to another plate of Chapman-Stone medium by spreading an area two by two centimeters. Six cultures will just fit on one plate. Incubate the plates exactly 48 hours at 30°C.

To apply the coagulase test [e.g., Dienst (1942)], collect as much as possible of the 48-hour growth into a loop and emulsify it in 0.10 ml. of Bacto brain heart infusion. Let it stand 15 minutes with frequent vigorous shaking, preferably by a shaking machine, and add 0.20 ml. of selected blood [Chapman (1945b) and Chapman (1946c)]. Incubate one hour in the incubator at 37°C. or in a water bath at 35°C. The average culture clots in 22 minutes but some may take an hour, rarely longer. They take an average of 40 minutes on staphylococcus medium No. 110 at 30°C.

To detect acid production from mannitol, add a drop of 0.04-per cent bromcresol purple to the cleared area left from the coagulase test. Any change in the color of the indicator, compared with that produced by an unused area similarly treated and examined promptly, indicates that mannitol has been fermented.

To use the Chapman-Stone medium in investigating a suspected outbreak of staphylococcus food poisoning, plate 0.01 ml. of several decimal dilutions of suspension of the suspected food and 0.10 ml. of a heavy fecal suspension, and rub the nose, throat, or other swab over the surface of the medium and spread the inocula with a glass spreader. Incubate the plates at 30°C. for exactly 48 hours. If the plates cannot be examined immediately they should be stored in sealed cans in the refrigerator, under which conditions they will remain suitable for testing for several days. Yellow or orange colonies that are surrounded by a clear zone probably are food-poisoning staphylococci. Apply the coagulase test and test for mannitol fermentation as described previously. If they are positive, the evidence is more convincing. Cultures may be tested for gastroenterotoxigenicity for further "confirmation" but, since it is generally agreed [Shaughnessy and Grubb (1937), Dack (1942), and Haynes and Hucker (1944)] that the presence of an almost pure culture of an orange, plasma-coagulating staphylococcus in large number in epidemiologically incriminated food is excellent presumptive evidence that they caused the outbreak, other tests are unnecessary for routine purposes.

SUMMARY

A culture medium is described which provides selective isolation of staphylococci and on which food-poisoning staphylococci produce pigmented colonies surrounded by a clear zone.

On this medium, staphylococci produce deeper pigment than by previous methods; they coagulate blood faster; they show the Stone reaction without having to flood the plate with ammonium sulfate solution; and fermentation of mannitol is determined, all on the original isolation plate. Transfer to another medium to check the results is only necessary when the number of colonies is small.

The medium should prove useful for the rapid routine testing of foods thought to be the cause of food poisoning.

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EFFECT OF OLD-FASHIONED AND MODERN METHODS OF COOKING ON THE RETENTION OF NUTRIENTS IN VEGETABLES. I. CABBAGE: INFLUENCE OF METHOD OF REPORTING DATA ON RESULTS[1]

MARY S. EHEART AND MATTIE LOU SHOLES
Department of Agricultural Chemistry, Virginia Agricultural Experiment Station, Blacksburg, Virginia

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For a number of years food specialists have emphasized the importance of improved methods of cooking vegetables. These improvements include shorter cooking periods, smaller quantities of water, and seasoning with salt and butter instead of the older methods by which vegetables were cooked for long periods of time in a moderate amount of water containing fat or fat meat. The reasons underlying these proposed changes were based on the chemical properties of the vitamins, such as water solubility, heat lability, or instability in alkaline solution. No study was found in the literature in which nutritive losses were studied from vegetables actually cooked by these old-fashioned methods. One study by Oser, Melnick, and Oser (1943) compared "old-fashioned" with "new-improved" methods of cooking vegetables but their old-fashioned methods did not simulate those usually thought of as old-fashioned in which vegetables were greatly overcooked. In their study the vegetables were cooked about the same length of time by the old-fashioned methods as by the new-improved methods but in a fairly large amount of distilled water.

A considerable amount of work has been done on the nutritive losses of vegetables cooked by modern methods but the results have shown wide variations. Two methods which have been studied to a great extent are (1) boiling in an open kettle in a sufficient amount of water to cover the vegetable, and (2) cooking in a tightly covered pan in the smallest possible amount of water to prevent scorching. In both methods the vegetable is cooked until just tender. Results for some previous work on cooking cabbage by these two methods are summarized (Table 1). It may be seen that the percentage retention of ascorbic acid varied from 30 to 54 for the open-kettle method and from 44 to 84 for cabbage cooked in a tightly covered pan. One cause for variation in results would appear to be due to the end point chosen for the time of cooking.

The importance of cooking vegetables to the same degree of doneness by different methods instead of cooking for the same length of time may be seen from the work of Van Duyne, Chase, and Simpson (1944), (Table 1), in which there was no difference in ascorbic acid retention when cabbage was cooked in four times its weight of water for 5.5 minutes and in twice its weight of water for 8.5 minutes. In much of the work reported in the

[1] Published with approval of the director of the Virginia Agricultural Experiment Station as scientific paper No. 8 from the Department of Agricultural Chemistry.

literature, the findings are based on vegetables cooked for the same length of time by different methods which would result in vegetables of different degrees of doneness. In the present study, cabbage was cooked until just tender by each of the modern methods.

TABLE 1

Summary of Previous Work on Losses of Ascorbic Acid From Cabbage Cooked in Open Kettle With Water to Cover and That Cooked in Tightly Covered Pan With Minimum Amount of Water

Method of cooking	Water to cabbage ratio	Cooking time	Ascorbic acid retained	Workers
		min.	*pct.*	
Open kettle	4.2:1	6-8	40	Brinkman, Halliday, Hinman, and Hamner (1942)
Open kettle	5 :1	10.0	32	Wellington and Tressler (1938)
Open kettle (Marion Market)	4 :1	9.5	34	Noble and Waddell (1945)
Tightly covered kettle (Marion Market)	Minimum	25.0	73	Noble and Waddell (1945)
Open kettle (Danish Ball Head)	4 :1	12.5	30	Noble and Waddell (1945)
Tightly covered kettle (Danish Ball Head)	Minimum	40.0	44	Noble and Waddell (1945)
Open kettle	4 :1	5.5	49	Van Duyne, Chase, and Simpson (1944)
Open kettle	2 :1	8.5	50	Van Duyne, Chase, and Simpson (1944)
Open kettle	5 :1	8.0	54	Ireson and Eheart (1944)
Tightly covered kettle	Minimum	9.0	84	Ireson and Eheart (1944)

The work reported here was undertaken to determine just what retention of nutrients actually results from the old-fashioned methods of cooking vegetables and the comparison of these methods with modern methods. The data for retention of nutrients by the old-fashioned methods should be valuable in the calculation of dietary studies in regions where it is known that these methods of cooking still prevail.

EXPERIMENTAL PROCEDURE

Statistical Design of Experiment:[2] Nine replications were made for each of five methods of cooking cabbage. The cabbage was also analyzed raw, thus making a total of 54 samples studied. The data were submitted to the analysis of variance for study.

An amount of cabbage sufficient for one complete replication was bought at one time from a local grocery store. This day-to-day variation (replication) in the nutritive value of cabbage bought from the market

[2] The statistical design of experiment was planned by Dr. Boyd Harshbarger and Dr. D. B. DeLury, statisticians in the Virginia Agricultural Experiment Station, and the analysis of the data was carried out under their supervision.

was desired in the experiment. Thus, for one cooking method to be better than another the difference would have to be evident in spite of the large daily variations in the nutritive value of cabbage as purchased by the average housewife. The heads of cabbage for each replication were cut into six to eight radial sections (depending upon the size of the head), the sections were mixed well, then samples were taken for analysis of the raw cabbage and for each method of cooking.

TABLE 2

Description of Cooking Methods Used

Method of cooking	Weight of cabbage cooked	Volume of water	Salt	Kind of fat used in cooking	Amount of fat	Average cooking time of samples	Method of preparation of cabbage
Tightly covered kettle	*gm.* 600	*ml.* 75	*gm.* 8.5	.....	.....	*min.* 18.4	Coarsely shredded
Open kettle	600	2,320	22.0	.....	.....	11.3	Coarsely shredded
Modified old-fashioned	650	1,200	8.5	Fat back	140	15.7	Radial sections, 2-3 inches outer thickness
Old-fashioned	810	2,100	8.5	Fat back	170	150.0	Radial sections, 2-3 inches outer thickness
Fried	730	600	11.5	Fryings from fat back	90	60.0	Chopped in ¼-½ inch pieces

Methods of Cooking: For some methods of cooking the cabbage was shredded or chopped; the amounts of cabbage used and other details of the cooking methods are given (Table 2). The method used for cabbage cooked in a tightly covered kettle was essentially that of the utility companies in which the coarsely shredded cabbage was cooked until tender with 75 ml. of water, an amount just sufficient to prevent scorching. The open-kettle method was essentially that of Halliday and Noble (1933) in which the coarsely shredded cabbage was boiled until just tender in an amount of water sufficient to cover the vegetable. The modified old-fashioned method was one recommended by the extension specialists which gave the meat flavor to the vegetables without overcooking them. The fat meat was cooked for two hours in the water prior to addition of radial sections of cabbage, which were boiled in this meat-water mixture until just tender.

The old-fashioned and fried methods were worked out with the help of extension specialists familiar with methods of cooking commonly used in the home. In the old-fashioned method the radial sections of cabbage, the fat meat, and a fairly large quantity of cold water were placed on a cold burner and cooked two and one-half hours. For the fried cabbage, so-called because it was seasoned with meat fryings, chopped cabbage was cooked in a moderate amount of water in a covered pan for 30 minutes, the fryings were added, the lid removed, and the cabbage was simmered 30 minutes longer. At the end of the cooking period the cabbage was drained, cooled, weighed, and samples were taken for ascorbic acid and mineral analyses

and for palatability tests. The cooking water was analyzed for ascorbic acid.

Analytical Methods Used: The samples for mineral analyses were dried in fruit jars in circulating air at 72°C.(161.6°F.). Fruit jars were used because large amounts of fat in some of the samples melted and would have been lost if paper bags had been used. The time required for drying samples by this method was sometimes as much as four or five days. After drying, the samples were weighed, ground in a hammer mill, and analyzed for moisture, ether extract, calcium, and phosphorus.

For moisture determinations small samples of the ground material were dried to constant weight in circulating air at a temperature of 72°C. Calcium and phosphorus were determined by the official methods of the Association of Official Agricultural Chemists (1940).

Because the amount of fat retained by the cabbage during cooking by several of the methods was almost as much as the dry matter in cabbage, it was necessary to analyze the samples for fat in order to report the nutrients on the true dry basis. The dried, ground samples were analyzed for ether extract with the Bailey-Walker extraction equipment according to the official methods of the Association of Official Agricultural Chemists (1940).

The cooking water and cabbage samples were analyzed for ascorbic acid by the Morell method (1941). A three-per cent solution of HPO_3 was used as the extractant and the amount of decolorization of indophenol dye, as obtained with the photoelectric colorimeter, was a measure of the amount of ascorbic acid present in the sample.

Judging the Cooked Cabbage: The cabbage to be judged for palatability was warmed immediately before serving. Three experienced judges scored the samples for color, texture, flavor, and aroma and the average scores were studied.

RESULTS AND DISCUSSION

It has been recognized by most workers in the field that the basis on which the nutritive value of vegetables is reported determines in a large measure the interpretation of results. Many workers feel that data are more comparable when reported on the dry basis, while others prefer the wet basis because food is consumed fresh. Most experiments in which cooking is involved report the data on the basis of losses incurred by the total cooked lot. In the present experiment the calcium, phosphorus, and ascorbic acid contents of cabbage are reported on all three bases—mg. per gram, dry basis; mg. per 100 grams, wet basis; and mg. per total cooked lot.

The calcium content of raw cabbage and of cabbage cooked by different methods is reported on the three bases (Table 3). Since a difference between two means was not considered of interest unless significant at the five-per cent level, values for least significant differences at this level are given (Table 3). When reported on the dry basis the calcium content of cabbage cooked by the old-fashioned method was significantly higher (4.79 mg. per gram) than the raw cabbage (3.82 mg. per gram), while lots cooked by the tightly covered kettle and fried methods were significantly lower than the raw, containing 3.22 and 3.25 mg. of calcium per

gram, respectively. On the wet basis the cabbage cooked by the old-fashioned method again was higher than the raw cabbage in calcium, containing 41.6 and 29.8 mg. per 100 grams, respectively. The lots cooked by the tightly covered-kettle and fried methods did not differ significantly from the raw. These lots contained 33 mg. per 100 grams for both methods. On the basis of total lots cooked the only significant change in the calcium content of the raw cabbage was the increase in calcium of that cooked by the old-fashioned method. This consistent increase in the calcium content of cabbage cooked by the old-fashioned method was obviously due to the long cooking period during which the cabbage was in contact with water which contained 45 parts per million of calcium. The absorption of calcium by vegetables cooked in tap water has been reported previously from this laboratory by Eheart and Sholes (1946).

TABLE 3

Calcium and Phosphorus Content of Cabbage Cooked by Different Methods When Reported on Three Bases

Method of cooking	Number of samples	Calcium content			Phosphorus content		
		Dry basis	Wet basis	Total lot[1]	Dry basis	Wet basis	Total lot[1]
		mg./gm.	*mg./100 gm.*	*mg.*	*mg./gm.*	*mg./100 gm.*	*mg.*
Raw	9	3.82	29.8	178.8	3.92	31.0	185.8
Tightly covered kettle	9	3.22	33.0	150.7	3.65	37.8	173.8
Open kettle	9	4.31	27.7	160.2	3.73	24.1	139.9
Modified old-fashioned	9	3.63	29.3	173.4	3.69	30.0	177.2
Old-fashioned	9	4.79	41.6	209.7	3.83	33.2	167.1
Fried	9	3.25	33.0	171.4	3.41	34.7	180.3
L.S.D.[2]	....	0.54	5.2	29.4	0.23	3.9	18.9

[1] Equivalent to 600 grams of raw cabbage. [2] Least significant difference at the five-per cent level.

If the open-kettle and tightly covered-kettle methods are compared, it may be seen (Table 3) that the former method was better in calcium retention when reported on the dry basis, whereas the latter method was significantly higher when the data were reported on the wet basis. When the two methods were compared on the basis of changes in the total lots, the difference between them was not significant.

A difference in results owing to the basis on which data were reported was also found true for the phosphorus content of cabbage cooked by different methods (Table 3). On the dry basis cabbage cooked in the tightly covered kettle contained 3.65 mg. per gram, a value significantly lower than that for the raw cabbage which contained 3.92 mg. per gram. On the wet basis cabbage cooked by this method was significantly higher in phosphorus than the raw (37.8 mg. per 100 grams of cooked cabbage compared with 31.0 mg. in the raw). On the basis of total cooked lots the difference between the raw cabbage and cabbage cooked by this method was not significant. The cabbage cooked in the open kettle was significantly lower than the raw in phosphorus when reported on the wet basis and on the basis of total lots but there was no difference when reported on the dry basis.

The most important nutrient of cabbage is probably its ascorbic acid content. Here again, results from the effect of method of cooking upon its retention depended upon the basis upon which the data were reported (Table 4). This effect was very obvious when the open-kettle and tightly covered-kettle methods were compared. On the dry basis the open-kettle method, with its use of a large volume of water, retained significantly more ascorbic acid during the cooking of cabbage than the tightly covered kettle, in which an amount of water just sufficient to prevent scorching was used. Cabbage cooked by the former method contained 4.34 mg. of ascorbic acid per gram, while that cooked by the latter method contained 3.58 mg. However, on the wet basis the tightly covered kettle gave a much higher retention of ascorbic acid than the open kettle since the cabbage cooked by the former method contained 37.1 mg. per 100 grams while that cooked by the latter contained only 28.1 mg. per 100 grams. On the total-lot basis in respect to ascorbic acid retention the difference between the two methods was not significant.

TABLE 4

Ascorbic Acid Content of Cabbage Cooked by Different Methods (When Reported on Three Bases) and Percentage Distribution of Ascorbic Acid in Cooked Cabbage, Cooking Water, and That Oxidized

Method of cooking	Number of samples	Ascorbic acid content			Distribution of ascorbic acid		
		Dry basis	Wet basis	Total lot[1]	Cooked cabbage	Cooking water	Oxidized
		mg./gm.	*mg./100 gm.*	*mg.*	*pct.*	*pct.*	*pct.*
Raw[2]	9	6.32	49.7	298.3	.....	.....	.....
Tightly covered kettle	9	3.58	37.1	170.7	56.9	.....	43.1
Open kettle	9	4.34	28.1	163.1	54.7	34.6	10.7
Modified old-fashioned	9	4.14	33.7	198.8	66.7	14.7	18.6
Old-fashioned	9	2.05	17.5	88.5	29.6	21.9	48.5
Fried	9	2.73	27.9	144.5	48.4	18.8	32.7
L.S.D.[3]	....	0.33	2.8	13.5	4.4	2.5	5.7

[1] Equivalent to 600 grams of raw cabbage. [2] The L.S.D. does not apply to the raw cabbage, which was obviously higher than all cooked samples. [3] Least significant difference at the five-per cent level.

The foregoing illustrations of differences in results owing to the different bases on which data are reported are sufficient to raise the question: which is the best basis to use in reporting data from cooking experiments?

When data are reported on the wet or per-serving basis, the values are affected by a loss or gain of water during the cooking of the vegetable. It may be seen (Table 5) that all methods of cooking except one caused a significant loss of weight (mostly water) during cooking, thus concentrating nutrients in the cooked cabbage. Cabbage cooked in the tightly covered kettle lost almost 150 grams during cooking (Table 5), thus producing cooked cabbage which was higher in both calcium and phosphorus than the raw cabbage when compared on the 100-gram wet basis. It is obvious that a method should not be considered better than others merely because it causes a large loss of water from the vegetable during cooking and an apparent high retention of nutrients. Data reported on the wet basis may

be of interest for calculation of dietaries but it seems inadvisable that this basis should be used in a comparison of methods of cooking.

Reporting data on the dry basis is becoming more prevalent. This method eliminates the problem of changes in water content of foods and is adequate for many types of studies but has its limitations in cooking experiments where seasonings are added to the vegetable during cooking. Salt and fat, if added, may be absorbed by the vegetable during cooking, thus apparently increasing its dry-matter content. In the present experiment the fried cabbage retained 112.5 per cent (Table 5) of the dry matter of raw cabbage. Since corrections for ether extract had been made on the dry-matter content of the samples, it was thought that the increase in dry matter during cooking was due either to loss of fat during the ether-extract determination or to absorption of NaCl by the cooked sample.

TABLE 5

Comparison of Cabbage Cooked by Different Methods for Cooked Weight, Retention of Dry Matter, Color, and Total Score

Method of cooking	Number of samples	Cooked weight[1]	Retention of dry matter of raw cabbage	Color[2]	Total score[3]
		gm.	*pct.*		
Tightly covered kettle	9	458.3	100.6	16.9	68.0
Open kettle	9	579.0	79.6	19.4	73.5
Modified old-fashioned	9	589.7	102.6	18.6	68.4
Old-fashioned	9	505.8	93.3	10.6	51.3
Fried	9	518.9	112.5	15.2	62.5
L. S. D.[4]	....	15.5	7.6	1.6	4.8

[1] Equivalent to 600 grams of raw cabbage. [2] Perfect score = 20. [3] Perfect score = 80. [4] Least significant difference at the five-per cent level.

A further study was made of the ether-extract method. There was a possibility that a significant amount of fat may have remained in the fruit jars containing the cooked samples with high fat content or that incomplete extraction of these samples may have resulted. Rinsing the fruit jars with ether proved that the error owing to fat remaining in the jars amounted to 1.14 per cent of the total ether extract, an error well within experimental limits. Re-extraction of the dried material gave no increase in ether extract. Thus, the method used for correction of fat absorbed during cooking was evidently satisfactory.

In order to determine whether or not absorption of NaCl during cooking was great enough to account for the increase in dry matter of the fried cabbage, all of the raw and fried samples (nine of each) were analyzed for total chlorides by the official method of the Association of Official Agricultural Chemists (1940). The difference between the chloride content of the raw and fried samples would obviously give the amount of NaCl absorbed during cooking. When the dry-matter content of these cooked samples was corrected for the NaCl absorbed, it was found that the percentage retention of dry matter of the raw was 98.1 instead of 112.5 or a loss of 1.9 per cent dry matter instead of a gain of 12.5 per cent.

Thus it would appear that if data are to be compared for cooking methods on the dry basis, corrections should first be made for ether extract (if fat has been added) and for NaCl absorption during cooking. Even after these corrections are made, the methods which retain the least amount of dry matter during cooking would give the highest apparent retention of nutrients. From all considerations, it would seem that data reported on the per-gram dry basis, or per 100 grams wet basis, have definite limitations. Thus, it appears that the most satisfactory method of reporting data from cooking experiments is on the basis of total cooked lots. Therefore, conclusions from the present experiment will be drawn from data for the total lots rather than for the per-gram dry basis or per 100 grams wet basis.

A comparison of results from the five cooking methods studied for retention of calcium during the cooking of cabbage showed that cabbage cooked in a tightly covered kettle, in an open kettle, fried, or cooked by the modified old-fashioned method did not differ significantly from the raw cabbage (Table 3). The cooked samples contained from 150.7 to 173.4 mg. per total cooked lot, whereas the raw cabbage contained 178.8 mg. Cabbage cooked by the old-fashioned method contained 209.7 mg. of calcium per lot, an amount significantly higher than in the raw cabbage, thereby indicating absorption of calcium from the water.

The phosphorus content of cooked cabbage did not vary significantly from the raw when cooked in a tightly covered kettle, by the modified old-fashioned method, by the old-fashioned method, or when fried (Table 3). Cabbage cooked by these methods varied from 167.1 to 180.3 mg. per cooked lot. However, cabbage cooked by the open-kettle method lost a significant amount of phosphorus, containing only 139.9 mg. per lot compared with 185.8 in the raw lot.

Cooking cabbage by all five methods caused a significant loss of ascorbic acid (Table 4). The raw cabbage contained 298.3 mg. in the 600-gram lot, whereas after cooking by the different methods only 88.5 to 198.8 mg. remained. The modified old-fashioned method yielded a cooked product significantly higher in ascorbic acid (198.8 mg. per lot) than all other methods. The tightly covered kettle and open kettle retained the next highest amounts but did not differ from each other significantly, showing retentions of 170.7 and 163.1 mg. per lot, respectively. Fried cabbage was next highest with 144.5 mg. in the cooked lots and the old-fashioned method, as was expected, was lowest with 88.5 mg. per lot. The percentage of the original amount of ascorbic acid found in the cooked cabbage, the cooking water, and that lost by oxidation varied from one method to another (Table 4). The cabbage cooked by the modified old-fashioned method retained 66.7 per cent of the original amount, whereas that cooked by the old-fashioned method retained only 29.6 per cent. A retention of 54.7 per cent was obtained by the open-kettle method, which was similar to results reported by Van Duyne, Chase, and Simpson (1944) and Ireson and Eheart (1944) (Table 1). The tightly covered kettle caused a retention of 56.9 per cent of the original ascorbic acid content, which was lower than generally reported (Table 1). Solution of the vitamin in the cooking water was greatest in cabbage cooked in the open kettle, 34.6 per cent, and least in the modified old-fashioned method, 14.7 per cent. Oxi-

dation of ascorbic acid was highest in cabbage cooked by the tightly covered-kettle method, 43.1 per cent, and by the old-fashioned method, 48.5 per cent, and was lowest in that cooked by the open-kettle method, 10.7 per cent.

The cabbage was judged for all factors of palatability, however, since differences in flavor, aroma, and tenderness were so small, statistical analyses were made only on color and total scores (Table 5). Cabbage cooked in the open kettle was judged highest in total palatability, 73.5 out of a possible score of 80, and that cooked in a tightly covered kettle or by the modified old-fashioned method was scored next highest, 68.0 and 68.4, respectively. The fried cabbage was given a total score of 62.5 and that cooked by the old-fashioned method, a low score of 51.3. Color was judged best in the cabbage cooked by the open-kettle and modified old-fashioned methods.

From all considerations, it appears that the method of cooking cabbage which has most to recommend it is the modified old-fashioned method. There is no loss of minerals by this method and the retention of ascorbic acid is higher than in the other methods. This cabbage rated second highest in palatability and gave the meat flavor so much preferred in many sections. This method should definitely be recommended over the old-fashioned and fried methods still commonly found in use. Any preference between the open-kettle method, in which a large quantity of water was used, and the tightly covered kettle, in which a small amount was used, cannot be justified on the basis of data herein reported. The retention of nutrients was approximately the same except for a greater loss of phosphorus by the open-kettle method. However, cabbage cooked by this method was judged higher in palatability than that cooked in the tightly covered kettle.

The good retention of nutrients by the modified old-fashioned method was thought to be due to the method of preparation of the cabbage, i.e., two- to three-inch radial sections. Therefore, it would seem advisable to recommend that cabbage be prepared in this manner and cooked in twice its weight of salted water, whether or not fat is added.

Although the mean squares of results obtained from the effect of method of cooking and replications are not given, it was found that in the case of both calcium and phosphorus contents, the daily variations (replications) were as great as the differences owing to methods of cooking. Although the daily variation in ascorbic acid content of cabbage was significant it was much lower than that owing to the method of cooking. Thus, the method used for cooking cabbage had a much greater effect on its ascorbic acid content than daily variations found in cabbage as purchased from the retail market. In the case of minerals the effects from method of cooking and daily variations were about the same.

SUMMARY AND CONCLUSIONS

Five methods of cooking cabbage—open-kettle, tightly covered-kettle, modified old-fashioned, old-fashioned, and fried—were compared for retention of nutrients during cooking and for palatability.

When data, reported on the per-gram dry basis, were compared with those on the 100-gram wet basis it was found that the conclusions differed as a result of several factors, such as loss of water during cooking and loss or gain of dry matter.

It was concluded from further study that the preferred method of reporting data from experiments on cooking vegetables was on the per-cooked lot basis. When this basis was used, the modified old-fashioned method was best for retention of ascorbic acid and should definitely be recommended over the old-fashioned and fried methods, both of which are still commonly used. Differences between the open-kettle method (using a large quantity of water) and the tightly covered kettle (using a very small quantity of water) were not great. Except for a greater loss of phosphorus and a higher palatability score by the open-kettle method there was no difference between the two methods.

Since the greater retention of ascorbic acid in the modified old-fashioned method was thought to be due to the method of preparation of the cabbage (two- to three-inch radial sections) it would seem from the present study that the best method to be recommended is as follows: cut cabbage into two- to three-inch radial sections with core included and cook in about twice its weight of salted water. The addition of meat for seasoning is optional.

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EFFECT OF VARIETY AND STORAGE ON ASCORBIC ACID CONTENT OF SWEET POTATOES

BOYCE D. EZELL, MARGUERITE S. WILCOX, AND MARGARET C. HUTCHINS

Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, Beltsville, Maryland

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Sweet potatoes are second in importance only to white potatoes as a vegetable crop in the United States. While they are generally considered mainly as a source of carbohydrates, they are a valuable source of vitamins as well. Vitamins are generally considered less stable than other food constituents. Vitamin C (ascorbic acid) is required in the diet in amounts greater than all the other vitamins combined, and is also one of the least stable. Since the rate of many biochemical reactions approximately doubles for each 10°C. rise in temperature, and since sweet potatoes are held for "curing" at a temperature near 29.4°C.(85°F.) for several days following harvest and may be stored for several months at temperatures around 12.8 or 15.6°C. (55 or 60°F.), a higher range than that recommended for most other fresh vegetables and fruits, the question of what happens to the ascorbic acid under these conditions is of considerable importance.

The results from previous studies along these lines are somewhat contradictory. Newton and Lowry (1937), using biological methods and the Porto Rico variety, reported that several months of storage had no appreciable effect on the vitamin C content. Scoular and Eakle (1943), using chemical methods and eight varieties and strains that had been stored four to six months, found that none of the varieties averaged over 2.75 mg. per 100 grams in the raw state and concluded that "sweet potatoes stored from four to six months at 50°F. and prepared by baking, boiling, and candying contribute an insignificant amount of vitamin C to the diet." Hollinger (1944), using seven varieties and selections, reported losses up to 39 per cent with varieties stored until May; and Speirs, Cochran, Peterson, Sherwood, and Weaver (1945), using a strain of Porto Rico, reported that losses amounted to 17 per cent during curing and that further losses in storage increased this amount to approximately 50 per cent in five or six months. With the exception of Scoular and Eakle (1943), whose low ascorbic acid values suggest that some uncontrolled factor may have affected their results, most workers agree that sweet potatoes, even those stored for several months, are an important source of vitamin C.

Sweet potatoes, in common with many other plant products, vary widely in ascorbic acid content in different samples of the same variety. Floyd and Fraps (1939) reported 15.4, 19.7, and 25.9 mg. per 100 grams, a difference of 68 per cent, for three samples of Porto Rico. Hollinger (1944) found a 72-per cent difference in five roots of the Triumph variety. Preliminary work by the present authors showed a range of 160 per cent in 30 individual roots of the Porto Rico variety. Unless adequate precautions

are taken to secure representative samples, the results may lead to erroneous conclusions.

Another factor that has sometimes received little attention is that of storage conditions. The roots used by Hollinger (1944) were stored in a room in which the temperature varied from 10 to 26.7°C.(50 to 80°F.). Whether her results would have been the same with a nearly constant temperature may be questioned. Sweet potatoes in storage are still living organisms, carrying on normal, though restricted, life processes, even to the initiation and development of adventitious buds and new plants. They are therefore subject to changes brought about by the normal functioning of these processes, which may be greatly modified by the storage conditions. A few degrees rise in temperature may favor increased metabolic activity and new growth, while a few degrees drop may subject the roots to physiological injury and subsequent attack from pathogenic organisms. A drop in temperature may be especially injurious if the roots are held even slightly below the minimum storage temperature recommended for sweet potatoes. Kimbrough and Bell (1942) studied the effects of low temperatures and concluded that the temperature should not be allowed to fall below 50°F. Harter and Weimer (1929) described an internal breakdown of sweet potatoes which they attributed to high temperatures combined with a relatively low humidity in storage, a condition usually resulting when the temperature of the storage room is permitted to rise. In the present study both temperature and humidity were mechanically controlled, and a greater number of roots were taken per sample than has been used heretofore.

MATERIALS AND METHODS

The sweet potatoes[1] used in these studies were grown at the Plant Industry Station, Beltsville, Maryland, in 1945. They were harvested in the forenoon, washed and placed in the curing room at 85°F. on the day harvested, and cured for eight or nine days. The cured roots were then carefully transferred to a storage room mechanically maintained at a temperature of 60°F. and a relative humidity of 85 per cent. Samples were analyzed before curing, shortly after curing, and at intervals during storage. Preliminary work during the preceding season showed that samples taken transversely from the center portion of the root gave results closely approximating those from the entire root. Roots divided into stem-end, center, and root-end sections averaged 16.20 mg. per 100 grams for the center section in comparison with 16.18 mg. for all three sections. Samples thereafter were taken from the center portion only. The ascorbic acid content of 30 individual Porto Rico roots determined in January varied from 15 to 39 mg. per 100 grams, fresh-weight basis, a range of 160 per cent. To insure that the results obtained were not due to individual root variations, 10 samples of five roots each were analyzed for each variety in each treatment in this study. The five unpeeled roots were split lengthwise, and one half of each root was used in sampling. Samples were taken in duplicate. In order to standardize and speed up sampling procedure

[1] The writers wish to express their appreciation to Dr. C. E. Steinbauer, in charge of sweet-potato investigations, for supplying the sweet potatoes used in this work.

and to reduce cut surfaces of the sample to a minimum, samples were taken with a cork borer. One cylinder of tissue, three-quarters of an inch in diameter, was taken at right angles to the long axis from the center portion of each root, and additional cylinders were taken as required to give a total of 50 grams of tissue. The weighed tissue was extracted in a Waring blendor according to the method of Loeffler and Ponting (1942), using 350 ml. of 0.4 per cent oxalic acid, Ponting (1943). The ascorbic acid content of the extract was determined in a photoelectric colorimeter and compensated for color and turbidity when necessary. Duplicate samples were averaged and the amount reported (Table 1) is the average of the 10 replicates. All results are reported on the fresh-weight basis at time of analysis.

RESULTS AND DISCUSSION

The ascorbic acid content of different varieties of sweet potatoes (Table 1) before curing ranged from 28.7 mg. per 100 grams for Porto Rico to 40.2 mg. for Nancy Hall. The latter variety was decidedly higher than the other varieties, while Southern Queen and Yellow Jersey were slightly but significantly higher than Triumph and Porto Rico. Samples taken shortly after curing showed that the different varieties had lost from 12 per cent of their original ascorbic acid for Unit 1 Porto Rico to 41 per cent for Yellow Jersey. The heavy loss in Yellow Jersey during the curing period reduced the ascorbic acid content below the other varieties, where it remained throughout the storage period. During curing and storage Yellow Jersey lost 42 per cent; Nancy Hall 36 per cent; Southern Queen and Porto Rico, 30 per cent; and Triumph, 26 per cent. Unit 1 Porto Rico lost 24 per cent by March 14, when the supply of that variety in this study was exhausted. Hollinger (1944) reported that varietal differences became less significant at the end of the storage period. In the present study Nancy Hall, the high variety at harvest, contained at that time 40 per cent more ascorbic acid than Porto Rico, the low variety. In April, when the tests were terminated, Nancy Hall contained 66 per cent more than Porto Rico. During storage Nancy Hall, still the high variety, averaged 28.6 mg. per 100 grams for the three storage periods, or 48 per cent more than the 19.2 mg. of the Yellow Jersey, the low variety in storage.

The maximum loss of ascorbic acid shown during curing and storage for each of the five varieties averaged 33 per cent. Only in the Porto Rico, however, did the lowest level occur at the end of the storage period. On the other hand, there was no significant decrease in the Yellow Jersey after the middle of November, nor in other varieties, except the Porto Rico, after January. In Yellow Jersey and Triumph the ascorbic acid content had increased slightly but not significantly in April, while in Nancy Hall and Southern Queen there were significant increases. The reason for these increases can only be surmised. To determine whether the initiation of new growth would have such an effect, roots of four varieties (our limited supply of Porto Rico prevented its being used) were transferred from 60°F. storage to 70°F., sprinkled with water, and kept covered with wet cloths to induce sprouting. Appreciable sprouting occurred in Yellow Jersey in 60°F. storage near the end of the season,

TABLE 1

Effects of Variety, Curing, and Storage on Ascorbic Acid Content of Sweet Potatoes

Treatment	Nancy Hall		Southern Queen		Yellow Jersey		Triumph		Unit 1 Porto Rico		Porto Rico	
	Date	Ascorbic acid	Date	Ascorbic acid	Date	Ascorbic acid	Date	Ascorbic acid	Date	Ascorbic acid	Date	Ascorbic acid
		mg./ 100 gm.		*mg./ 100 gm.*		*mg./ 100 gm.*		*mg./ 100 gm.*		*mg./ 100 gm.*		*mg./ 100 gm.*
Before curing	10/16	40.2	10/15	32.6	10/18	32.4	10/19	29.5	10/23	29.8	10/22	28.7
Shortly after curing	11/15	30.6	11/14	28.3	11/16	19.1	11/19	24.6	11/26	26.2	11/20	24.2
After storage at 60°F	1/2	25.9	1/8	22.8	1/4	18.9	1/5	22.4	.....	.....	1/7	24.5
After storage at 60°F	3/6	26.2	3/13	23.5	3/7	19.0	3/8	21.8	3/14	22.6	3/12	20.9
After storage at 60°F	4/8	33.6	4/23	27.0	4/9	19.7	4/15	22.6	.....	.....	4/25	20.2
Average, 5 sampling dates	.....	31.3	.....	26.8	.....	21.8	.....	24.2	.....	.....	.....	23.7
One week at 70°F	.....	.....	.....	.....	3/25	15.4	.....	.....	.....	.....	.....	.....
Two weeks at 70°F	.....	.....	.....	.....	4/1	19.6	.....	.....	.....	.....	.....	.....
One month at 70°F	4/17	35.2	4/19	28.2	.....	.....	4/18	28.7	.....	.....	.....	.....
Difference for significance:												
Between storage periods												
5-per cent level	.....	2.0	.....	2.1	.....	1.1	.....	1.7	.....	1.6	.....	1.3
1-per cent level	.....	2.7	.....	2.8	.....	1.5	.....	2.3	.....	2.3	.....	1.7
Between variety averages												
5-per cent level	.....	1.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1-per cent level	.....	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

but no significant change in ascorbic acid took place at that temperature. With the other varieties only an occasional sprout occurred at 60°F. At 70°F. the Yellow Jersey sprouted abundantly in one week, and in two weeks the surface was thickly covered with young shoots. About a month was required for sprouting to become general in the other varieties.

In determining the ascorbic acid content of the sprouted roots the young shoots were removed and discarded before sampling so that the ascorbic acid found was present in the roots and not in the young, actively growing shoots. However, the tissue forming the adventitious buds before they break through the surface and the immediately adjoining cells are active. With vigorously sprouting roots these tissues might include a considerable part of the root. It may also be expected that the storage cells are more active when starch conversion is taking place (either to supply energy and materials for the growth of the new shoots or for other metabolic activities) than they are during the period in which the stored starch remains intact. In general, ascorbic acid tends to be more concentrated in the more active tissues, so one might logically expect an increase in the area involved in the development of the young shoots, and to a lesser degree perhaps in those cells in which starch conversion is taking place even though no sprouts are developing. The results obtained agree in general with this hypothesis.

In Nancy Hall variety there was a significant increase in ascorbic acid between the March and the April samplings. Little or no sprouting occurred during this period. Starch conversion apparently was taking place rather rapidly, however, as indicated by the fact that many of the roots were beginning to get pithy at the latter date, and a large part of them were showing a tendency for the skin to separate from the flesh and slip if oblique pressure was applied to the root, an indication of senescence. There was a similar but smaller increase in ascorbic acid in Southern Queen without the accompanying indications of senescence. However, while both Nancy Hall and Southern Queen showed a few sprouts on most of the roots after one month at 70°F., these were much fewer in number than in the other varieties. In neither of these varieties was the increase in ascorbic acid, at the higher temperature, significantly greater than at 60°F. when no sprouting took place. This is probably due to the tendency of sweet potatoes to form the first sprouts near the stem end of the roots. Samples taken from the middle section of the roots, as was done throughout this study, were therefore outside the immediate area affected by the sprouts. This tendency to form the first sprouts near the stem end of the root, outside the area from which the samples were taken, might also explain in part why no increase in ascorbic acid was found in Yellow Jersey at 60°F. although appreciable sprouting took place. When this variety was placed at 70°F., the number of sprouts increased greatly within one week, and in two weeks the surface was thickly covered with young shoots. At the end of the first week there was a sharp drop in the ascorbic acid content, but at the end of two weeks it had increased by an equal amount. The extent of the drop and the subsequent increase is statistically highly significant, far greater than can be accounted for by

sampling error. The reason for this behavior is not definitely known; but if ascorbic acid is utilized in respiration and in various enzyme systems, as has been frequently suggested, the increased metabolic activity in the rapidly sprouting roots at the higher temperature may have caused a temporary reduction in ascorbic acid at first, followed, however, by an increase within two weeks. Had our supply of roots permitted another sample to have been taken later it is possible that a further increase, above the 60°F. lot and similar to that found in Triumph after one month, would have been found. It should be remembered that Yellow Jersey sprouted much more quickly at 70°F. than did the other varieties and was sampled after one week and after two weeks at this temperature. The other varieties required a month for sprouting to become general and were sampled at the end of that period. Only in the Triumph and the Yellow Jersey did abundant sprouting occur over the entire surface of the roots. At 60°F. Triumph showed no significant increase in ascorbic acid at the April sampling over that in March; but in the lot held for one month at 70°F. sprouts and feeding roots thickly covered the surface and the ascorbic acid content had increased by 27 per cent during the month.

From a nutritional standpoint it is of considerable interest that, with the exception of Porto Rico, no appreciable loss in ascorbic acid occurred after January, under storage conditions prevailing in the present work. Instead, an increase occurred in Nancy Hall and Southern Queen between the March and the April sampling dates. This increase is probably associated with increased metabolic activity of the still living roots. While the increase in ascorbic acid doubtless occurs at the expense of other stored materials, it does enhance the value of the stored sweet potatoes as a source of vitamin C. The fact that Porto Rico continued to lose ascorbic acid while the other varieties remained stationary or increased, would suggest varietal differences in response to storage temperatures. Storage conditions may also influence the rate of loss of ascorbic acid and whether or not an increase will occur. This phase of the study is being continued.

The ascorbic acid content of these five varieties of sweet potatoes on the five dates of sampling, extending from the time of harvest in October until late April, averaged 25.5 mg. per 100 grams of fresh tissue. Sherman (1946) in his recent book states that "fresh raw tomatoes from various sources, analyzed in different laboratories have averaged about 23 milligrams per 100 grams of the edible portion." Sale *et al.* (1946) reported that the ascorbic acid content of 100 samples of tomatoes as delivered to 34 juice factories located in various parts of the United States averaged 24.4 mg. per 100 ml. of juice. Speirs *et al.* (1945) reported that "neither boiling nor baking whole sweet potatoes caused any loss of ascorbic acid." It would seem, therefore, that sweet potatoes are equal to tomatoes as a source of vitamin C. In addition they are superior, according to Sherman (1946a) to tomatoes as a source of provitamin A, calcium, magnesium, manganese, potassium, sodium, phosphorus, chlorine, copper, sulphur, iron, protein, and fats. Sherman's median values for thiamin and riboflavin are 36 and 63 per cent, respectively, higher in sweet potatoes than tomatoes; and sweet potatoes surpass even white potatoes as a source of carbohydrates.

SUMMARY

The ascorbic acid contents of Nancy Hall, Southern Queen, Triumph, Porto Rico, and Yellow Jersey sweet potatoes were determined at harvest in October, shortly after curing (November), and in January, March, and April during the storage season. The averages for the different varieties ranged from 21.8 mg. per 100 grams fresh weight in Yellow Jersey to 31.3 mg. in Nancy Hall, with a mean of 25.5 mg. per 100 grams. Sweet potatoes thus contain about the same amount of ascorbic acid, on a fresh-weight basis, as do tomatoes and may therefore be classed as a good source of vitamin C.

There is a significant difference between varieties in the vitamin C content. Of the five varieties used in this study Nancy Hall was the highest and averaged 43 per cent more than Yellow Jersey, the lowest variety.

Losses of ascorbic acid during the curing period ranged from 12 to 41 per cent in different varieties and averaged 22 per cent. Maximum losses during curing and in storage ranged from 26 to 42 per cent and averaged 33 per cent.

In most varieties used in this study the minimum ascorbic acid content occurred before the end of the storage season. In some of the varieties there was an increase in late storage.

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VARIATION IN COMPOSITION OF WINTER SQUASHES[1]

ARTHUR D. HOLMES, C. TYSON SMITH, AND
WILLIAM H. LACHMAN
Massachusetts Agricultural Experiment Station,
Amherst, Massachusetts

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For more than 300 years squashes have been an important article of the human diet in this locality, particularly during the fall and winter months. In his early writings about the foods of the Indians, Roger Williams (1643) refers to their use of squashes, and Safford (1917) reported that the prehistoric inhabitants of the Americas included squashes in their diet. This extremely long use of squashes for human food amply attests to their popularity and gives empirical approval of their dietary value. However, in spite of this long use of squashes, the data regarding their nutritive value is not complete. Accordingly, the purpose of this study was to assemble additional information concerning the composition of winter squashes, particularly with respect to individual variation within different varieties.

EXPERIMENTAL PROCEDURE

Five varieties of squashes were included in the study—Blue Hubbard (*Cucurbita maxima* L), Buttercup (*Cucurbita maxima* L), Butternut (*Cucurbita moschata* L), Des Moines (*Cucurbita pepo* L), and Golden Cushaw (*Cucurbita moschata* L). These five varieties were chosen because they are among the most widely used winter squashes in this general area. The squashes were grown on an alluvial soil reported to be a modified drift that accumulated from the waning ice sheet. According to Emerson (1897) the area is the bottom of the Hadley post-glacial lake; Beaumont (1943) classified the glacial out-wash material as Merrimac fine sandy loam. The seed was planted the first week in June and the squash plants were fertilized with a commercial 5-10-5 fertilizer at the rate of 2,000 pounds per acre. The squashes were harvested the first week in October. During the season the squashes were subjected to climatic conditions reported by Gunness (1946) to be 1,100 hours of bright sunshine, 17 inches of rain, and average monthly temperatures of 65, 70, 66, and 64°F., respectively, for June, July, August, and September. Owing to unforeseen conditions, it was impossible to assay the squashes for six weeks after they were harvested. During that period they were stored upon plant benches in greenhouses. The average greenhouse temperature was 70°F., and the squashes were exposed to 250 hours of bright sunshine. Edible portions of the five varieties of squashes were assayed for water, nitrogen, reducing sugars, carotene, ascorbic acid, calcium, magnesium, phosphorus, and potassium. The carotene content of the squashes was determined by the method of Wall and Kelley (1943), as modified by Holmes and Spelman (1946), and

[1] Contribution No. 600, Massachusetts Agricultural Experiment Station.

TABLE 1

Variation in Composition of Winter Squashes (Fresh Basis)

Common name	Water	Protein[1]	Reducing sugars	Carotene	Ascorbic acid	Calcium	Magnesium	Phosphorus	Potassium
Blue Hubbard	*pct.*	*pct.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
1	83.0	1.7	2.50	1.7	16.3	12	24	44	235
2	85.2	1.2	2.73	3.7	16.9	15	18	26	220
3	86.7	1.2	3.38	3.7	9.2	18	21	19	248
4	88.6	1.5	4.31	3.8	5.8	30	18	17	219
5	90.3	0.8	4.06	4.1	9.2	11	14	18	142
Average	86.8	1.3	3.40	3.4	11.5	17	19	25	213
Buttercup									
6	73.1	1.6	4.17	3.7	47.0	14	28	47	424
7	77.0	1.0	4.66	3.3	41.0	12	19	56	411
8	77.2	1.3	3.43	3.0	54.6	12	26	59	413
9	78.5	1.3	5.23	3.4	26.8	20	20	42	322
10	79.3	1.6	3.84	4.6	28.5	17	21	39	496
Average	77.0	1.4	4.23	3.6	39.6	15	23	49	413
Butternut									
11	79.1	1.2	0.74	6.9	7.4	26	29	49	543
12	81.1	1.2	0.57	9.7	7.9	18	24	57	558
13	83.2	0.9	0.65	7.4	4.8	25	25	65	455
14	84.2	1.4	1.07	7.9	4.8	15	20	36	480
15	84.8	0.7	1.19	11.8	3.8	21	21	55	399
Average	82.5	1.1	0.84	8.5	5.7	21	24	52	487
Des Moines									
16	84.2	1.7	1.17	1.3	4.7	33	39	31	461
17	85.3	1.3	1.26	0.9	3.7	33	36	23	423
18	88.2	1.0	1.17	0.6	4.5	28	30	22	440
19	88.7	1.0	1.20	0.9	2.5	32	28	19	365
20	89.0	1.5	1.80	0.7	1.5	31	27	18	356
Average	87.1	1.3	1.32	0.9	3.4	31	32	23	409
Golden Cushaw									
21	81.6	2.0	0.34	8.3	16.1	12	23	49	622
22	82.8	2.5	0.53	5.5	15.2	16	25	76	637
23	84.1	1.6	0.51	4.5	19.9	12	20	40	551
24	84.2	1.9	0.50	3.5	16.4	17	21	63	518
25	84.5	1.6	0.62	5.6	15.8	10	20	46	552
Average	83.4	1.9	0.50	5.5	16.3	13	22	55	576

[1] Protein = nitrogen × 6.25.

the amounts of the other components were determined by the official methods used by the Association of Official Agricultural Chemists (1940).

RESULTS AND DISCUSSION

Water: The average water content in the different varieties ranged from 77 per cent for Buttercup to 87.1 per cent for Des Moines. These values are definitely lower than 84.5 per cent reported by Yeager and Latzke (1932) for mature Buttercup squashes and 90.61 per cent reported by Holmes and Spelman (1946) for Blue Hubbard after winter storage. The latter authors also obtained values of 87 and 89.4 per cent of water for Butternut and Golden Cushaw, respectively, for squashes after winter storage as compared with the values of 82.5 and 83.4 per cent found in this study. In fact, Holmes, Spelman, and Jones (1945) found only 84.61 per cent of water in immature Golden Cushaw squashes that were being used as summer squashes. Although the water content of the individual squashes varied somewhat, the agreement within the variety was fairly close since the maximum values for Blue Hubbard, Buttercup, Butternut, Des Moines, and Golden Cushaw were 108, 108, 107, 107, and 102 per cent, respectively, of the minimum values.

Protein: The maximum average value for protein, 1.9 per cent, was found in the Golden Cushaw, whereas the minimum, 1.1 per cent, was found in the Butternut squashes. The variation in protein content of individual squashes within a variety was much greater than the variation in water content. The maximum amounts in Blue Hubbard, Buttercup, Butternut, Des Moines, and Golden Cushaw were 212, 160, 200, 170, and 156 per cent, respectively, of the minimum amounts of protein. At harvest time Phillips (1946) found 0.9 per cent of protein in Blue Hubbard, 1.3 per cent in Buttercup, and 1.2 per cent in Butternut squashes; and Culpepper and Moon (1945) reported that Blue Hubbard contained 1.56 per cent of protein and Des Moines 1.69 per cent. Yeager and Latzke (1932) found 1.13 per cent of protein in Buttercup squashes.

Reducing Sugars: The results of assays of the squashes for reducing sugars were variable. The lowest value was 0.5 per cent for Golden Cushaw and the largest was 4.23 per cent for Buttercup. The maximum values for the reducing sugars for the Blue Hubbard, Buttercup, Butternut, Des Moines, and Golden Cushaw were 162, 152, 161, 154, and 182 per cent, respectively, of the minimum values. Phillips (1946) found 2.98 per cent total reducing sugars in Blue Hubbard, 2.02 per cent in Buttercup, and 1.21 per cent in Butternut. Yeager and Latzke (1932) report 5.72 per cent of reducing sugars in Buttercup squashes. Thus the values obtained in this study are intermediate between those of Phillips (1946) and Yeager and Latzke (1932).

Carotene: The Des Moines squashes contained the smallest quantity of carotene, i.e., 0.9 mg. per 100 gm., and the Butternut squashes contained the largest amount, 8.5 mg. per 100 gm., or nearly 10 times that of the Des Moines squashes. The Golden Cushaw squashes were also a rich source of carotene since they contained an average of 5.5 mg. per 100 gm. This value was somewhat lower than that of 6.21 mg. per 100 gm. reported by Holmes, Spelman, and Jones (1945) for immature Cushaw, and materially

lower than that reported by Holmes and Spelman (1946) for Golden Cushaw at the end of winter storage, namely, 7.12 mg. The latter authors also found 2.22 mg. of carotene per 100 gm. in Blue Hubbard and 7.94 mg. in Butternut squashes at the end of winter storage.

Reduced Ascorbic Acid: The average ascorbic acid content of the five varieties of squashes varied from 3.4 mg. per 100 gm. for Des Moines to 39.6 mg. for Buttercup, the latter being more than 10 times the former. Whereas the Des Moines contained the least amount of both carotene and ascorbic acid, the Butternut contained the largest amount of carotene and the Buttercup the largest amount of ascorbic acid. The amount of ascorbic acid in the individual squashes was quite variable for each of the five varieties studied.

Calcium: The Des Moines squashes contained definitely more calcium, 31 mg. per 100 gm., than did the other four varieties. The values obtained for the individual Des Moines squashes were quite consistent since the maximum value was only 115 per cent of the minimum value, the amount in four of the squashes varying by only two mg. per 100 gm. The Golden Cushaw contained 13 mg. and the Blue Hubbard 17 mg. of calcium per 100 gm. as compared with 16 and 19 mg., respectively, previously reported by Holmes and Spelman (1946) for these varieties at the end of winter storage.

Magnesium: The maximum amount of magnesium observed in this study, 32 mg. per 100 gm., was found in the Des Moines squashes. Since all the varieties of squashes were grown under the same soil, fertilizer, and climatic conditions, it appears that the Des Moines has a greater capacity for depositing calcium and magnesium in its tissues than do the other four varieties studied. The average magnesium content of Blue Hubbard, Butternut, and Golden Cushaw, 19, 24, and 22 mg. per 100 gm., respectively, is significantly larger than the values of 11, 15, and 8 mg. per 100 gm. found by Holmes and Spelman (1946) in the respective varieties of squashes in the early spring after they had been held in storage since the previous harvest season.

Phosphorus: The average phosphorus content of the Des Moines and Blue Hubbard was similar, being 23 and 25 mg., respectively. The Butternut and Golden Cushaw, which have many similar characteristics, contained similar amounts of phosphorus, i.e., 52 and 55 mg. per 100 gm., respectively, the latter values being somewhat more than twice those of the former. The values obtained in this study for the phosphorus content of Blue Hubbard, Butternut, and Golden Cushaw in early winter storage are in closer agreement but in general less than the values found by Holmes and Spelman (1946) at the end of winter storage, i.e., 34, 68, and 29 mg. per 100 gm. However, the latter values were obtained for squashes not grown under uniform conditions.

Potassium: The Blue Hubbard squashes contained 213 mg. per 100 gm. of potassium. Essentially the same values, 413 and 409 mg. per 100 gm., were obtained for the Buttercup and the Des Moines squashes. The Butternut and Golden Cushaw squashes, which have several identical characteristics, contained much more potassium than the Blue Hubbard, Buttercup,

or Des Moines; but the average values obtained for them, 487 and 576 mg. per 100 gm., were not in as close agreement as had been anticipated.

SUMMARY

Assays of the edible portion of five varieties of squashes commonly used in this area as a winter vegetable for water, protein, reducing sugars, carotene, reduced ascorbic acid, calcium, magnesium, phosphorus, and potassium showed considerable variation. Blue Hubbard was not as rich in carotene phosphorus, and potassium as Butternut and Golden Cushaw, which are relatively new varieties that are gaining popularity. The Buttercup squash, which is frequently referred to as "a dry squash," contained less water and more reducing sugars than any of the other varieties. The Butternut was very rich in carotene, phosphorus, and potassium. Des Moines contained the smallest amount of carotene and ascorbic acid but the largest amount of calcium and magnesium of any of the varieties. Golden Cushaw was rich in carotene and contained the most protein, phosphorus, and potassium of any of the varieties included in this study. The results of the assays show considerable variation in the composition of the different varieties of winter squashes and of the different squashes within the varieties even though all were grown under uniform soil, fertilizer, and climatic conditions.

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OBSERVATIONS ON SPANISH SHERRY PROCESS

IRENE BRAJNIKOFF[1] AND W. V. CRUESS[2]
Food Technology Laboratory, University of California, Berkeley, California

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Jerez de la Frontera, a Spanish town near Cadiz, is the center of the Spanish sherry industry, and the English word "sherry" is probably derived from the Spanish word "Jerez." Spanish sherry differs from the Californian in that it is not cooked or baked artificially nor "cooked" in the sun, and it undergoes several years' treatment with "flor," a peculiar film-forming yeast. A further difference is use of the solera system in Spain, in which several layers or rows of casks of the wine are maintained permanently "in series"; that is, the last layer or row of casks contains the oldest wine, and the first row or layer the youngest wine. There may be six or more layers of casks in series forming the solera.

The film yeast, or flor, is growing continuously on the wine in each cask which is filled only about four-fifths full. Normally a portion, said to be 25 per cent, of the wine from the oldest row or layer of casks is removed about twice a year and fortified. The wine so removed is replaced by wine from the preceding row or layer. This layer, in turn, is replenished from the preceding, and so on to the new wine in the first row or layer, or to the "criadera" (new wine cellar). Some of the soleras are very old. The casks are never emptied unless they become leaky; hence there is a heavy accumulation of yeast that forms in the bottom of the casks as the film breaks and settles.

The yeast film is highly oxidative at the surface, but it abstracts oxygen from the wine in the depths of which conditions are strongly anaerobic during film growth.

Another peculiarity of the Spanish sherry process is "plastering" of the freshly crushed grapes and juice; i.e., the addition of burned gypsum (plaster of Paris). This lowers the pH value by converting much of the cream of tartar into free tartaric acid and acid potassium sulfate.

During the flor or solera stage the wine is maintained at about 15 to 15.5 per cent of alcohol by volume because at much below 15 per cent vinegar bacteria can develop and above 16 per cent the film yeast is less active.

The Palomino variety of grape is generally used. Crushing and pressing operations are rather primitive, but the greatest skill and care are used throughout the life of the wine in the solera.

During the past 12 years investigations have been conducted in this laboratory and by one of the authors (Cruess) in a number of California wineries, with the purpose of establishing the Spanish methods in California in order to improve the quality of our sherries.

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[1] Former graduate student in Food Technology, University of California.

[2] Professor of Food Technology and Biochemist in the Experiment Station, University of California.

In 1938, Cruess, West, and Gililland reported on preliminary results of investigations one the use of Spanish film yeasts in making sherry under California conditions. Hohl and Cruess (1939) reported on the properties of 15 strains of flor yeasts from Jerez, Spain, and from the Arbois district of France. The typical flor yeast closely resembles ordinary wine yeast, *Saccharomyces ellipsoideus*, except for its ability to form a film on wine. De Castella of Australia (1909) has given a very good account of the making sherry by the flor and solera system in Jerez.

TABLE 1

Effect of "Flor" Yeasts on Volatile Acidity in Wine of Low Initial Volatile Acidity

Yeast No.....	2	3	4	5	7	11	13	14	15	16
Time	Volatile acidity as acetic (grams per 100 c.c.)									
mo.										
0	.054	.054	.054	.054	.054	.054	.054	.054	.054	.054
1	.054	.054	.054	.054	.054	.054	.054	.054	.054	.054
2	.054	.049	.049	.054	.048	.054	.054	.049	.054	.049
3	.048	.045	.042	.048	.040	.040	.054	.048	.036	.042
4	.042	.042	.042	.060	.042	.036	.036	.036	.042	.033
5	.048	.036	.036	.054	.036	.030	.030	.042	.036	.024
6	.042	.033	.036	.048	.030	.036	.026	.036	.033	.024

The present paper presents the results of recent laboratory scale investigations on the flor yeasts and on "plastering." The film yeasts used were University of California Nos. 2, 3, 4, 5, and 7, Spanish sherry yeasts; and Nos. 11, 13, 14, 15, and 16, French yeasts from the Arbois region, also termed "Chalôn" yeasts for a famous winery, Chateau Chalôn.

Film Formation: Yeast No. 13 (French) formed a film very slowly in wine of 15.5 per cent alcohol. Nos. 2 and 16 formed a film rather slowly, while the others formed films rapidly and at similar rates. All except No. 13 formed heavy films on three-gallon lots of wine, and these films broke and sank to the bottoms of the five-gallon demijohns several times during six months' storage at room temperature. All of the wines developed a pungent odor and characteristic flavor.

Effect on Volatile Acidity: In the preceding experiment the initial volatile acidity of the wine was 0.054 gram per 100 c.c. as acetic. Volatile acidity determinations were made once a month throughout the six months' period, with the results shown (Table 1).

On the average, the volatile acidity was reduced to about three-fifths of the original. Yeasts No. 2 and 5 reduced the volatile acidity less than did the others, and Yeasts No. 13 and 16 more than did the others.

In another experiment white wine of 15.5 per cent alcohol was brought to high volatile acidity, 0.1939 gram per 100 c.c., by addition of acetic acid. Lots of about 700 c.c. each were pasteurized in capped quart bottles. The caps were replaced with sterile cotton plugs and the wines inoculated with pure cultures of the film yeasts. They were stored at room temperature and analyzed for volatile acidity at weekly intervals, with the results shown (Table 2).

Yeast No. 13 did not develop a film during the experiment. As is usually the case with small lots of wine, the rate of acid destruction was very rapid; the rate appears to vary more or less directly with the ratio of unit surface area to depth. Most of the samples were reduced to extremely low volatile acidity.

TABLE 2

Effect of "Flor" Yeasts on Volatile Acidity of Wine of High Initial Volatile Acidity

Yeast No.....	2	3	4	5	7	11	13	14	15	16
	Volatile acidity as acetic (grams per 100 c.c.)									
mo.										
0	.194	.194	.194	.194	.194	.194	.194	.194	.194	.194
2	.194	.182	.161	.178	.182	.194	.194	.194	.194	.194
3	.176	.097	.097	.146	.169	.170	.194	.194	.194	.182
4	.152	.096	.096	.066	.060	.098	.194	.164	.155	.146
5	.066	.078	.092	.039	.054	.015	.194	.054	.066	.042
6	.030	.009	.015	.012	.030	.012	.194	.012	.012	.012

The destructive effect of the film yeast on volatile acidity is of practical importance in that if for any reason acetic bacteria gain the upper hand for a short time and raise the acetic acidity to a dangerous level, it is possible to arrest acetification by fortification to 15 to 16 per cent alcohol and destroy the excess acetic with the flor film.

In another experiment it was found that none of the 10 strains of film yeast would develop on white wine of 0.246 gram volatile acidity per 100 c.c. Five grew and five failed to grow in the same wine at 0.224 gram volatile acidity. All grew at 0.200 gram. The period of observation in all cases was 30 days.

In a winery experiment with a 1,000-gallon oval of white wine, the film yeast maintained growth at about 0.217 gram volatile acid per 100 c.c., and slowly reduced the volatile acidity. Undoubtedly most of the drop in volatile acidity is due to oxidation of the acetic acid to CO_2 and H_2O, although considerable ethyl acetate is formed in some cases.

Effect on Total Acidity: In the three-gallon lots of white wine previously mentioned, the rates of lowering of total acid were observed as shown (Table 3).

In our winery experiments the decrease in total acidity was very slow. For example, at the Italian Swiss Colony winery two 120-gallon butts of wine inoculated with flor yeast No. 5 contained 0.39 and 0.50 gram total acid on May 2, 1940; on April 27, 1943, the values were 0.34 and 0.39. Results were similar with 18 other butts in this same winery.

In another winery two 50-gallon barrels of aged wine inoculated with yeast No. 5 contained 0.585 and 0.517 gram of total acidity on September 20, 1938; on May 14, 1940, the values were 0.48 and 0.46. Similar data were obtained for about 100 other wines under winery conditions.

With small lots, one gallon or less, the total acidity was reduced in 12 months in most cases to below 0.20 gram per 100 c.c.; with several smaller lots of 300 to 500 c.c. it was reduced to less than 0.10 gram per 100 c.c., or practically to 0.

To separate portions of white wine, tartaric acid was added to give 0.54, 0.75, 1.00, 1.23, and 1.50 grams of total acidity. These were pasteurized and inoculated with film yeast and held at room temperature. At the end of four weeks the total acidities had become 0.437, 0.625, 0.885, 1.15, and 1.36 grams per 100 c.c., indicating a fairly uniform rate of oxidation at the various acidities. Similar results were obtained with wines acidified with citric and lactic acids. Also, the total acidity of orange wine was reduced by the yeast. The data indicate that these three acids and acetic acid are oxidized by the flor yeast.

TABLE 3

Effect of Film Yeasts on Total Acidity

Yeast No.....	2	3	4	5	7	11	13	14	15	16
Time	Total acidity as tartaric (grams per 100 c.c.)									
mo.										
0	.435	.435	.435	.435	.435	.435	.435	.435	.435	.435
1	.421	.417	.417	.417	.405	.421	.435	.405	.405	.417
2	.398	.406	.386	.412	.386	.409	.435	.402	.394	.402
3	.397	.394	.371	.402	.364	.386	.435	.398	.318	.386
4	.345	.330	.338	.375	.338	.338	.352	.338	.308	.352
5	.322	.308	.340	.345	.300	.352	.338	.322	.308	.338
6	.322	.292	.278	.330	.292	.338	.330	.315	.292	.338

Alcohol Tolerance: To verify the results reported by Hohl and Cruess, since their data were obtained about 10 years ago with the freshly isolated yeasts, experiments were repeated to determine the approximate alcohol tolerance of the film stage of 10 strains. Most of the yeasts grew at 16.5 per cent, but none grew at 17 per cent; yeast No. 14 grew at 15 per cent but not at 16 per cent; and yeast No. 13's tolerance was below 15 per cent. By gradual increase in alcohol content by small increments at two-week intervals, yeast No. 5 was acclimated to 17 per cent alcohol. In a winery experiment the film has continued to grow in several butts of wine at 16.7 per cent alcohol. These results confirm those of Hohl and Cruess.

SO_2 Resistance: SO_2 tolerance of the flor yeasts is of interest since this compound is used to prevent the growth of spoilage bacteria in wines. Unexpectedly, film formation occurred within four weeks by No. 5 and No. 14 in wine to which 350 p.p.m. of SO_2 had been added shortly before inoculation. This level is about three times that used to hold spoilage bacteria in check. These observations confirm observations made in winery experiments with 50- to 110-gallon lots of wine in which growth readily occurred at 150 to 175 p.p.m. of SO_2, and in three barrels at 300 p.p.m.

Effect of pH Value on Flor Yeast Activity: The pH values of small lots of about 700 c.c. each of wine of 15.5 per cent alcohol were brought to the pH values shown (Table 4) by adding citric acid or NaOH. Volatile acidity determined at the intervals shown in the table was used as the criterion of yeast activity. Yeast No. 5 was used.

At pH 2.65 there was no growth until near the end of the experiment, indicating that it is near the limit of growth. The rates of destruction

TABLE 4

Effect of pH Value on Volatile Acid Destruction

pH.....	3.95	3.6	3.36	3.2	3.0	2.85	2.65
Time	Volatile acidity as acetic (grams per 100 c.c.)						
days							
0	.066	.066	.066	.066	.066	.066	.066
14	.024	.024	.030	.030	.030	.036	.063
19	.015	.018	.021	.021	.021	.027	.063
26	.014	.015	.012	.015	.015	.018	.060
47	.....	.....	.....	.....	.....	.....	.024

after the film was established were similar for the various pH values.

Effect of Flor on pH Value: The pH value of the previously mentioned 10 lots of three gallons each of wine on which were growing the various flor yeast strains were determined at monthly intervals. The initial pH was 3.75; the data on pH changes is summarized (Table 5).

TABLE 5

Changes in pH Value of Wine by Action of Ten Strains of Flor Yeast

Yeast No.....	2	3	4	5	7	11	13	14	15	16
Time	pH values									
mo.										
0	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
1	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
2	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
3	3.80	3.80	3.80	3.80	3.80	3.75	3.75	3.80	3.80	3.78
4	3.75	3.80	3.80	3.80	3.85	3.78	3.78	3.80	3.85	3.80
5	3.85	3.90	3.95	3.95	3.97	3.90	3.87	3.95	3.96	3.87
6	4.08	4.15	4.15	4.13	4.13	4.10	4.11	4.15	4.15	4.10

In general, the yeasts induced similar rates of increase in pH, this increase being due to a corresponding decrease in total acidity occasioned by oxidation.

Effect of Flor Yeast on Sugar in Wine: It is of interest to know whether the film stage of the yeast utilizes sugar which may be present in the wine in rather low concentration.

Yeast No. 5 was inoculated into approximately 700 c.c. of wine containing 15.5 per cent alcohol, 0.675 gram sucrose, and 0.57 gram of invert sugar per 100 c.c. The cultures were stored at room temperature.

At the end of 10 days the sucrose content had dropped to 0.314 gram per 100 c.c. and the invert to 0.50 gram per 100 c.c. At 21 days there remained 0.01 gram sucrose and 0.24 gram invert sugar per 100 c.c. On the 27th day there was no sucrose and 0.23 gram of invert sugar per 100 c.c.

In another experiment 10 strains of the flor yeasts destroyed considerable of the one gram per 100 c.c. of added dextrose per 100 c.c. in 40 days at room temperature.

Formation of Esters and Aldehydes: Ten strains of film yeast were grown for six months on three-gallon lots of wine of 15.5 per cent alcohol as previously mentioned. Esters and aldehydes were determined at four, five, and six months (Table 6).

TABLE 6

Ester and Aldehyde Formation by Film Yeasts

Yeast No.....	2	3	4	5	7	11	13	14	15	16
Time	Esters (mg. per 100 c.c.)									
mo.										
4	20.1	18.5	19.4	18.5	17.6	17.6	17.6	17.6	17.1	18.5
5	24.6	20.2	20.7	20.2	18.5	18.5	21.1	18.0	17.6	20.2
6	28.2	20.3	21.1	20.3	19.3	19.3	25.5	18.5	18.5	22.0
	Aldehydes (mg. per liter)									
4	127.0	51.0	52.5	127.2	44.5	38.7	55.4	55.4	37.7	37.7
5	138.2	59.0	55.3	130.9	49.7	41.5	65.4	63.5	39.6	38.7
6	148.2	61.2	61.2	153.0	55.3	52.6	79.0	69.1	41.5	44.2

Yeasts No. 2 and 5 produced more aldehyde than the other yeasts, a condition reflected in the flavor as the two wines acted upon by their films possessed more of the characteristic Spanish fino flavor than did the wines from other yeasts.

Effect of Oxygen Supply: Three portions of 3,000 c.c. each of white wine of 15.5 per cent alcohol were inoculated with the film stage of yeast No. 5. The containers were plugged with cotton and the film allowed to develop at room temperature for one month. One container was then corked tightly; one was corked with a rubber stopper with a $\frac{1}{16}$-inch hole, plugged with cotton, and the other was left with its original cotton plug in place.

After several months at room temperature the wine in the container with the cotton plug carried a heavy film and was yeasty in flavor. The wine in the tightly corked container had dropped most of its film and had developed considerably more "fino" flavor and bouquet than the other two. That in the container with cork stopper with the $\frac{1}{16}$-inch hole was intermediate. Other experiments of like character, as well as observations in winery tests, tend to confirm the result observed here, namely, that after the film is established air supply should not be too abundant if a yeasty flavor is to be avoided.

Effect of Added $CaSO_4$: Calcium sulfate was added to 900 c.c. portions of dry white wine about one year old in amounts ranging from 0 to 32 grams per 1,000 c.c., and allowed to stand at room temperature. The bottles were shaken once a day for a week. The pH value was affected very little; that of the untreated wine was 3.60, and that of the wines with two to 32 grams added $CaSO_4$ per 1,000 c.c. had dropped to pH 3.45 only, in all cases. The corresponding sulfate contents as SO_4 were 0.043 and 0.106 gram per 100 c.c., the latter probably being chiefly $CaSO_4$.

When added to fresh juice before fermentation the results were as shown (Table 7), the analyses being on the new wine after fermentation.

There was a marked increase in tartness and H-ion concentration when up to eight grams of $CaSO_4$ per liter was added. The $CaSO_4$-treated wines cleared more rapidly after fermentation and were paler in color than the check. The lowered pH would tend to hold spoilage bacteria in check.

In several other experiments eight grams of $CaSO_4$ was added to the must with beneficial effect on pH value and flavor. As previously mentioned, its addition to the fresh juice is regular practice in Spain.

TABLE 7

Effect of $CaSO_4$ Added Before Fermentation

$CaSO_4$ (gm. per liter)	0	1	2	4	8	16	32
pH value	3.8	3.7	3.45	3.35	3.25	3.20	3.20
Total sulfates as SO_4	0.023	0.136	0.205	0.279	2.282	0.314	0.330

The reason that $CaSO_4$ has so little effect on pH value, if added to aged wine, is that the wine contains very little bitartrate, $KH(C_4H_4O_6)$, whereas the fresh juice is supersaturated with this substance. It crystallizes out in the wine during fermentation and during the first few months of aging. The change in pH on addition of $CaSO_4$ is induced by conversion of bitartrate to free tartaric acid as follows: $CaSO_4 + 2KH(C_4H_4O_6 \rightarrow Ca(C_4H_4O_6) + K_2SO_4 + H_2(C_4H_4O_6)$. The added $CaSO_4$ had but little effect on the rate of fermentation of the must.

Composition of Commercial Sherries: Four samples of well-known brands of California baked sherries made without flor yeasts and of five imported Spanish sherries were analyzed; the results are given (Table 8).

TABLE 8

Composition of Several California and Spanish Sherries

California sherries	Total acidity as tartaric	Volatile acidity as acetic	Alcohol volume	pH value	Total sugars	Total ash	Sulfates as SO_4
	gm./100 c.c.	*gm./100 c.c.*	*pct.*		*gm./100 c.c.*	*gm./100 c.c.*	*gm./100 c.c.*
Solano Winery dry sherry	.39	.064	18.85	3.65	1.40	.236	.064
CrestaBlanca dry sherry	.42	.048	19.20	3.58	2.14	.289	.082
Concannon Calif. sherry	.58	.067	18.70	3.55	2.42	.374	.144
Calif. Growers' Calif. sherry	.45	.054	18.80	3.45	2.42	.308	.107
Spanish sherries							
Amoroso	.48	.085	20.7	3.47	4.93	.401	.158
Pinta	.46	.054	21.0	3.40	1.84	.462	.221
Amontillado	.48	.066	19.6	3.30	2.38	.428	.252
Oloroso	.53	.085	20.0	3.4	6.95	.408	.202
Apitiv	.33	.048	19.3	3.75	1.08	.371	.182

The Spanish sherries, on the average, were slightly lower in pH than the Californian and noticeably higher in total ash and sulfates. The Amoroso and Oloroso samples were sweet sherries for which there were no California counterparts. In other respects the two groups of sherry were comparable in composition.

SUMMARY

The processes of making California and Spanish sherries differ chiefly in that flor (film) yeasts and the solera system are used in Spain and the wine is not baked, whereas in California the wine is baked for several months, and neither flor yeasts nor the solera system are employed.

The film stage of flor yeasts oxidizes acetic (volatile) acid as well as the fixed acids of wine.

Below 15 per cent alcohol acetification is likely to occur during "flowering" (the film stage), and at or above 17 per cent alcohol the film usually fails to form. For practical purposes its maximum alcohol tolerance is about 16.7 to 16.8 per cent by volume. Recommended alcohol content for use of the film yeast is 15.5 to 16.5 per cent.

SO_2 resistance of the film stage is high; above 300 p.p.m. total SO_2.

It also tolerates low pH (high acidity) growing readily at pH values of 3.95 to 2.85. At 2.65 initial growth was slow, but eventual development good, this pH apparently being near the lower limit of growth.

The flor yeast reduces the total acidity slowly in large lots of wine exposed to air and rapidly in small lots, causing corresponding increases in pH.

Residual small amounts of sugar or small amounts of added sucrose and invert sugar are destroyed rather rapidly by the film stage.

The 10 strains of yeast studied formed comparable amounts of esters, but there was a considerable range of production of aldehyde. The maximum final aldehyde content was 153 p.p.m. for yeast No. 5, and the minimum was 41.5 p.p.m. for yeast No. 15.

After the film was well established through abundant air supply better flavor was developed in small-scale experiments with limited air supply.

$CaSO_4$ added to wines had very little effect on pH, but when added to the juice before fermentation, it reduced the pH very markedly and correspondingly increased the tartness of taste by conversion of bitartrate to free tartaric acid and acid potassium sulfate. (See text for explanation.)

Several Spanish sherries from the San Francisco market were similar to California sherries of like class (dry versus sweet) in total acidity, volatile acidity, and alcohol content. The Spanish sherries were somewhat lower in pH and much higher, on the average, in total ash and sulfates.

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LYSOZYME ACTIVITY OF REHYDRATED, SPRAY-DRIED, WHOLE-EGG POWDER

S. E. HARTSELL[1]

The Bacteriological Laboratories
Department of Biology, Purdue University,
West Lafayette, Indiana

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While studying the bacteriology of stored, spray-dried, whole-egg powder, answers were sought to several questions relating to the probable lysozyme content of the powder. Could lysozyme titers of the rehydrated eggs be determined and, if so, under what conditions? Would such titers correlate with the total bacterial count of the powder when stored? Were lysozyme-susceptible bacteria still present in the stored powder? What would be the extent of the thermostability of the lysozyme in rehydrated, spray-dried, whole eggs? This report concerns the data now at hand to assist in answering these questions.

The isolation and qualitative measurement of lysozyme from egg white has been recorded by many investigators, but its activity when mixed with egg yolk has been the subject of few experiments. Fleming (1929) stated that egg yolk had no lytic action, while a 1 to 60,000,000 dilution of egg white had some lytic activity on cocci. Cabezas and Rubina (1942) found that egg yolk contains a small amount of lysozyme but when it is mixed with egg white the bacteriolytic power decreases. No other reports have been found wherein the lytic activity of liquid whole eggs (mixed yolks and whites) has been recorded. For convenience of expression throughout this report, the lytic activity of the rehydrated liquid whole eggs was regarded as due to lysozyme, therefore, the term—lysozyme titer—is used. This interpretation was prompted by the work of Thompson (1940) who wrote: "Provisionally it seems justifiable to include the lytic actions of lysozyme-containing materials on various saprophytes as evidence of lysozyme action."

EXPERIMENTAL PROCEDURE AND RESULTS

Could lysozyme titers be determined on rehydrated eggs? An 11-gm. sample of the whole-egg powder was ground in a sterile mortar and pestle. The entire contents of a 90-ml. phosphate buffer dilution blank (pH 6.4)[2] was added and the sample rehydrated. Serial dilutions were then made in the buffer diluent. One milliliter of each dilution was added to one ml. of a heavy suspension (MacFarland nephelometer No. 8) of *M. lysodeikticus* which had been suspended in phosphate buffer. These mixtures were incubated in a water bath at 52°C.(125.6°F.). Lysis was noted by clearing of

[1] The author wishes to express his appreciation for the technical assistance of Miss Julia Steffey in carrying out this study. Very valuable suggestions concerning the manuscript were given by Dr. George Stewart, Iowa State College, Ames, Iowa.

[2] Unpublished experiments with rehydrated, purified lysozyme have shown this diluent, Sorensens' buffer, to be preferable to physiological saline or distilled water when testing lytic activity on *M. lysodeikticus.*

the suspension. The lysozyme titer of the sample was regarded as the reciprocal of the highest dilution of the rehydrated eggs that gave complete lysis of the cell suspension in six hours.

The following table shows a typical titration for a sample of fresh, spray-dried, whole-egg powder that was rehydrated in the manner described.

TABLE 1

Lysozyme Titration of Rehydrated, Whole-Egg Powder

Dil. of lysozyme	Time				
	15 min.	30 min.	1 hr.	4 hr.	6 hr.
Sample No. 10					
10 T[1]	3+	4+	4+	4+	4+
100 T	/	2+	3+	3+	4+
200 T	0	1+	1+	2+	4+
400 T	0	0	0	1+	3+
800 T	0	0	0	0	1+
1 M	0	0	0	0	0
Control[2]	1+	2+	4+	4+	4+

[1] T = thousand; 10 T, therefore, is a 1 to 10,000 dilution of the rehydrated egg powder; 0 = no lysis, / = trace, 1+ = slight lysis, 2+ = moderate lysis, 3+ = nearly complete lysis, 4+ = complete lysis. [2] The control was a 1 to 1,000,000 dilution of egg-white lysozyme from fresh eggs diluted in the same manner as the sample.

Nineteen samples of fresh powder tested in this way gave essentially similar results, titers of 200,000. This observation was expected since the method of testing is, at best, only roughly quantitative and would not detect very slight differences in lysozyme content in the samples. It is interesting to note that in the higher dilutions of the sample longer periods of time were necessary before macroscopic evidence of lysis was seen. Continued incubation of the 1 to 1,000,000 dilution of the sample for 18 hours did not result in complete lysis of the cell suspension. Obviously, the rapidity and amount of lysis are directly proportional to the concentration of lysozyme in the sample.

Would such titers correlate with the total bacterial count of the powder as storage continues? Spray-dried, whole-egg powder, both compressed and loose-packed, was stored at 0, 10, 20, and 37°C. (32, 50, 68, and 98.6°F.), Hartsell (1944). When samples were taken for the determination of bacterial counts, lytic activity was also measured in the same manner used for the fresh powder; results of this phase of the study are presented (Table 2).

TABLE 2

Lysozyme Titer in Stored Egg Powder

Temperature of storage	Initial titer	Titer after storage period of		
		1 month	2 months	3 months
°C.				
0	200,000	200,000	200,000	200,000
10	200,000	200,000	200,000	200,000
20	200,000	100,000	100,000	100,000
37	200,000	40,000	40,000	40,000

The lysozyme titers were the same for loose-packed egg powder and compressed egg powder. No differences were noted in the lysozyme titers for powder packaged in tin cans, in grease-proof packages, or in carbon-liner packages.

It is interesting to note that the higher the temperature of storage the lower the lysozyme titer in the rehydrated powder. The lytic activity was rapidly reduced at 37°C.; however, it was not completely lost during the three months' storage period. Since the total bacterial count dropped sharply as the powder was stored at 37°C., the direct relationship of lysozyme titer and total bacterial count is obvious. This statement does not mean that the lysozyme contributed to the reduction of bacteria in the product after drying; it is improbable that lysozyme could do so in the absence of moisture. The factors of dessication and storage temperature are far more significant than lytic activity in reducing the numbers of viable bacteria in the egg sample.

The explanation for the loss in lytic activity as the powder was stored is difficult. Recently, denaturation of the proteins in liquid whole eggs was studied by Payawal, Lowe, and Stewart (1946). Certain observations made by them are pertinent; they wrote:

> "There are at least eight heat-denaturable proteins in egg white; among them are albumin (1 and 2), conalbumin (1 and 2), globulin (1, 2, and 3), and mucin. In addition, the yolk contains several others, namely lecitho-vitelline, cephalo-vitelline, and livetin, according to Cruickshank (1941), Jukes and Kay (1932), and Longsworth, Cannon, and MacInnes (1940).
>
> "Our knowledge concerning the heat-denaturation phenomenon for the various proteins is very limited. Even if we knew a great deal about the pure components, the behavior of the natural mixture in yolks, whites, and whole eggs could not be predicted with complete certainty."

They were successful, however, in using viscosity, as determined by a capillary viscometer, to study the effect of heat treatments on denaturation of liquid whole eggs.

It is probable that the loss in lytic activity noted in the above experiments was not due to denaturation but to the insolubility of the proteins in the dried, whole-egg powder. Stewart and Kline (1941) and Stewart, Best, and Lowe (1943) have shown that solubility stability is correlated with the dextrose in the albumen, the pH of the eggs before and after drying, and the storage temperature.

When the dextrose was removed by fermentation of the albumen, stabilization of the solubility occurred. These effects were attributed, in part, to a lowering of the pH of the whole egg. Controlled adjustment of the pH of whole egg, prior to drying, contributed to the retention of solubility during storage of the dried product. The adjustment of the pH of the whole egg was not possible in the present study; however, it was assumed that approximately optimal conditions for the rehydration and subsequent lytic activity of lysozyme, were provided when a buffer of pH 6.4 was used as a diluent.

Stewart, Best, and Lowe (1943) observed that as the temperature of storage of dried egg powder was increased the solubility decreased. The rate of these changes was greatly accelerated at temperatures above 30°C.

(86°F.). The rapid loss in lysozyme titers of the powder held at 37°C. in these experiments makes the loss in solubility a more appropriate explanation for the effects noted.

Were lysozyme-susceptible bacteria still present in the egg powder? The bacteria isolated from the spray-dried, whole-egg powder stored for three months, Hartsell (1944), were tested for lysozyme susceptibility with homologous lysozyme—that is, the sample was rehydrated and diluted with phosphate buffer in the manner indicated previously, and the isolations *from that sample only* were subjected to its lysozyme (Table 3).

TABLE 3

Lysozyme Susceptibility of Bacteria Isolated From Stored, Spray-Dried, Whole-Egg Powder

	Microorganism	Lysis		Microorganism	Lysis
	Achromobacter litorale	—		*Flavobacterium acetylicum*	—
3[1]	*Bacillus cereus*	—	2	*Flavobacterium devorans*	+
	Bacillus megatherium	—		*Flavobacterium fulvum*	—
3	*Bacillus mesentericus*	—		*Flavobacterium radiatum*	+
2	*Bacillus simplex*	—	2	*Flavobacterium rhenii*	—
10	*Bacillus subtilis*	—	2	*Micrococcus candicans*	—+
3	*Bacillus tumescens*	—	5	*Micrococcus candidus*	(1)—(4)+
2	*Bacillus ubicuitarius*	—	3	*Micrococcus cinnebarius*	—
3	*Bact. erythrogenes*	—		*Micrococcus flavescens*	+
6	*Bact. globiforme*	—	4	*Micrococcus freundreichii*	(1)—(3)+
8	*Bact. linens*	—	2	*Micrococcus luteus*	+
2	*Bact. parvulum*	—	2	*Micrococcus saccatus*	—+
2	*Flavobacterium aurantiacum*	+		*Sarcina lutea*	—
				Sarcina ventriculi	—

[1] Numbers refer to the frequency of isolation from different samples of egg powder; + = lysed by homologous lysozyme, — = no lysis. Numbers in parentheses indicate the number of strains that gave that result.

It is evident that more (19) lysozyme-resistant strains were obtained from stored egg powder than lysozyme-susceptible strains (9). This result was expected since many of the bacteria surviving the storage conditions were bacilli. The literature records that few species of bacilli are susceptible to lysozyme. Furthermore, lysozyme has been shown to have more activity at a pH of 6.4, Epstein and Chain (1940), which point in the pH scale may not be optimal for the solution of the proteins in the cells of bacilli. Lysozyme may have affected the strains of bacilli but lysis may not have been possible under the conditions of these experiments.

Epstein and Chain (1940) showed that when the proteins in the cells of *M. lysodeikticus* were denatured by heat, complete lysis did not occur in the presence of lysozyme; only partial lysis was noted. Perhaps a similar denaturation of the proteins in the cells of the bacteria in the liquid eggs occurred during the drying process, thus altering their susceptibility to the action of lysozyme. Epstein and Chain believed that egg-white lysozyme acts on other saprophytes by the same mechanism that it attacks *M. lysodeikticus*. However, the phenomenon of lysis appears to be a complex reaction involving bacterial enzymes as well as lysozyme, Meyer, Thompson, Palmer, and Khorazo (1936). The variable results obtained with the isolations in this study might be explained on this basis.

That saprophytic cocci are susceptible to lysozyme has been repeatedly recorded in the literature. The occurrence of both susceptible and resistant species of micrococci and sarcina in the egg powder is difficult to explain. Perhaps these species were present when the liquid eggs were stored in the cold-wall tanks and, prior to drying, the lysozyme destroyed the very susceptible cells; therefore, only lysozyme-resistant cells survived or the micrococci and sarcina were introduced into the product during processing and handling and were not subjected to the lytic action of the lysozyme until their exposure in these tests. The factor of denaturation noted above may have influenced the lysis of these strains. The identification of the substrate in the cells of the micrococci that is necessary for lysozyme activity would need to be done for conclusive evidence, Meyer and Hahnel (1946).

As a point of special interest, the isolations from fresh egg-powder samples were subjected to the lytic action of the homologous lysozyme (Table 4).

TABLE 4

Lysozyme Susceptibility of Bacteria Isolated From Fresh, Spray-Dried, Whole-Egg Powder

	Microorganism	Lysis		Microorganism	Lysis
	Achromobacter guttatum	+		*Leuconostoc* species	+
	Aerobacter aerogenes	—	5	*Micrococcus candidus*	(4)—(1)+
3[1]	*Bacillus cereus*	—		*Micrococcus conglomeratus*	+
10	*Bacillus subtilis*	—		*Micrococcus perflavus*	+
3	*Bacterium erythrogenes*	—		*Micrococcus piltonesis*	+
	Flavobacterium lucanatum	+		*Micrococcus viscosis*	+
3	*Leuconostoc mesenteroides*	+		*Sarcina citrea*	—
				Staphylococcus epidermidis	+

[1] Numbers refer to the frequency of isolation from different samples of egg powder; + = lysed by homologous lysozyme, — = no lysis.

Resistance of the bacilli and susceptibility of the saprophytic cocci are again evident. The data record the effect of lysozyme on several saprophytic bacteria hitherto untested for lysis (Tables 3 and 4). Fleming (1929) and Thompson (1940) refer to the effect of lysozyme on saprophytes but list very few species upon which it has been tested.

What would be the extent of thermostability of the lysozyme in rehydrated, spray-dried, whole-egg powder? Since the liquid eggs were dried rapidly it was thought that very slight denaturation of the proteins took place immediately and that if changes were brought about upon storage, the lysozyme content of the product would be affected. Data recorded previously in this report showed this to be the case. Experiments were then conducted to see what effect additional exposure to heat would have on the lysozyme titer of diluted, rehydrated powder. One ml. of a 1 to 10,000 dilution of the lysozyme in rehydrated fresh powder was exposed in test tubes in a water bath at the selected temperature for 30 minutes. One ml. of a suspension of *M. lysodeikticus* was then added and the mixture incubated at 52°C.(125.6°F.) for six hours. Temperatures above 85°C.(185°F.) were not used since the diluted, rehydrated, whole eggs

became murky, thereby preventing the reading of lytic activity; the results of a typical test are recorded (Table 5).

TABLE 5

Thermostability of Lysozyme in Rehydrated, Spray-Dried, Whole-Egg Powder

Temp. of exposure	Lysis of *M. lysodeikticus* after exposure				
	15 min.	30 min.	1 hr.	4 hr.	6 hr.
°C.					
50	4+	4+	4+	4+	4+
60	4+	4+	4+	4+	4+
70	4+	4+	4+	4+	4+
80	0	2+	4+	4+	4+
85	0	2+	3+	4+	4+

Apparently the lysozyme of rehydrated, whole-egg powder is quite thermostable; however, under the conditions of these experiments temperatures above 70°C.(158°F.) retarded its activity. Fleming (1929) found that egg-white lysozyme was destroyed after 30 minutes at 75°C. (167°F.). Meyer, Thompson, Palmer, and Khorazo (1936) state that lysozyme is very stable toward heat and acid. Linz (1937) observed that purified lysozyme from egg white is not inactivated at 100°C.(212°F.) when dissolved in weakly acidified physiological saline. The retarding effect of 85°C. noted in these experiments may be due to the fact that whole eggs were used and phosphate buffer (pH 6.4) was the diluent. The question then arose—could the lytic activity in the diluted rehydrated powder be destroyed after prolonged heating at 85°C.?

The same conditions were provided to expose a 1 to 10,000 dilution of the lysozyme for varying periods of time at 85°C. The *M. lysodeikticus* suspension was then added and lysis noted for an observation period of six hours.

TABLE 6

Thermostability of Lysozyme at 85°C.(185°F.)

Time of exposure	Time of lysis of *M. lysodeikticus*				
	15 min.	30 min.	1 hr.	4 hr.	6 hr.
min.					
5	2+	4+	4+	4+	4+
15	1+	4+	4+	4+	4+
30	0	2+	4+	4+	4+
45	0	/	3+	4+	4+
60	0	0	2+	4+	4+
90	0	0	2+	4+	4+

The rapidity of lytic activity of the diluted, rehydrated egg powder is directly proportional to the time of exposure at 85°C. The activity is still evident, though retarded, after 90 minutes of exposure at this temperature. Some inhibiting action may have been due to the proteins in the whole-egg powder, or to additive effects of proteins and phosphate buffer; however, no information is at hand on these reactions.

SUMMARY

Lytic activity, apparently owing to lysozyme, has been observed by rehydrating spray-dried, whole-egg powder with phosphate buffer at pH 6.4 and exposing *M. lysodeikticus* to its action. Lysozyme titers decreased as the temperature of storage increased. Apparently, the basic polypeptide that is lysozyme was altered, or its solubility affected, as storage of the powder continued. Lytic activity was still evident after exposure of diluted, rehydrated powder to 85°C. for 90 minutes. Under the conditions of these experiments, no difference could be detected in the lysozyme titers of spray-dried, whole-egg powder packed in tin cans, carbon-liner packages, or grease-proof packages. Likewise, compressed or loose-packed powders gave equivalent lysozyme titers. Many more lysozyme-resistant bacteria are present in stored egg powder than in the fresh egg powder. The probable factors affecting the evidence of lysis on the strains are discussed.

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VITAMIN CONTENT OF SNAP BEANS GROWN IN SOUTH CAROLINA

FRANCES R. HAYDEN, P. H. HEINZE, AND B. L. WADE
U. S. Regional Vegetable Breeding Laboratory, Charleston, South Carolina

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Snap beans, whether grown for fresh marketing, processing, or home use, are an important crop in many sections of the country. The commercially canned pack of snap beans ranks next in size to those for tomatoes, peas, and corn. The raw beans contain appreciable amounts of thiamin, riboflavin, carotene, and ascorbic acid, although processing, cooking, and serving somewhat diminish these quantities. Different values for these vitamins in raw snap beans have been reported. These values are difficult to compare because investigators have worked with varieties from many seed sources; the crops were grown in various localities under different environmental conditions; and the snap beans were not always analyzed at similar stages of maturity by the same laboratory procedures.

At the U. S. Regional Vegetable Breeding Laboratory at Charleston, South Carolina, an increase in the vitamin content is one of the objectives in the program of breeding snap beans adapted to the region. In this report varieties and strains of bush snap beans were analyzed for ascorbic acid, thiamin, and riboflavin in the spring and fall of three consecutive years. In addition, carotene content was determined during one season.

MATERIALS AND METHODS

Samples of snap beans for vitamin studies were taken from strains grown in replicated yield tests with commercial varieties. At Charleston snap beans are planted twice each year, about the middle of March for the spring crop and about the middle of August for the fall crop. Single-row plots, 32 x 3 feet, seeded 75 beans per plot, were used at all times. The snap beans were picked at random along the rows in the early morning and washed in distilled water before being analyzed. Beans were sorted and pods not in good condition for table use were discarded. The blossom and stem ends were removed and the whole beans cut into one-inch lengths for chemical determinations. Forty-gram samples were used for ascorbic acid analyses as described by Heinze, Kanapaux, Wade, Grimball, and Foster (1944). The extraction was made with one per cent metaphosphoric acid, and the decolorization of the indophenol dye was measured by an Evelyn photoelectric colorimeter. The ascorbic acid analyses were completed the day the beans were harvested. The extracts for thiamin and riboflavin determinations were prepared either the day the beans were harvested or on the following day from beans stored overnight in a refrigerator. Carotene samples were weighed, quick-frozen in sealed cellophane bags, stored at —17.8°C.(0°F.), and analyzed several weeks later.

The preparation of the extract and the methods of analysis for thiamin and for riboflavin were the same as those given by Poole, Heinze, Welch,

and Grimball (1944) for cabbage, i.e., a modification of the Conner and Straub (1941) procedure for thiamin determination and of the Mackinney and Sugihara (1942) procedure for riboflavin determination. Starting in the fall of 1944 and during the following seasons, the enzyme preparation, Pectinol, was included with the other enzyme materials, taka-diastase and papain, for the digestion of the thiamin and riboflavin extracts. Also starting in the fall of 1944 and thereafter, riboflavin was determined by direct measurement of fluorescence of the filtered extract according to the method of Peterson, Brady, and Shaw (1943). Sodium hydrosulfite was used to destroy fluorescence of the riboflavin and obtain a "blank" for each solution. The fluorescence of the final solutions in the thiamin and riboflavin determinations was measured by a Coleman Model 12 photofluorometer.

With some modification the procedure described by Wall and Kelley (1943) was followed for the analysis of carotene. Before extracting the blended alcohol-ether mixture with petroleum ether, 100 ml. of 30-per cent potassium hydroxide solution was added to the mixture in the separatory funnel. This made unnecessary the addition of sodium sulfate to help separate the aqueous and ether layers. A No. 440 filter was used in the Evelyn photoelectric colorimeter for reading the solutions. Dry-matter content was determined on 20- to 25-gram samples dried 20 hours at 70°C. (158°F.) in a forced-draft oven. The data were treated statistically and significant differences between means were determined according to methods given by Snedecor (1940).

RESULTS

Spring 1943

A survey of 171 varieties and strains of snap beans at marketable stage was made in the spring of 1943 for ascorbic acid, thiamin, riboflavin, and dry-matter content. About 16 strains of snap beans were harvested and analyzed each week day from May 26 through June 11. Data from these analyses are presented (Table 1); mean values for the strains are classified according to their ancestry, although all are several selected generations beyond the original crosses. The snap beans averaged 20.3 mg. of ascorbic acid, 71 micrograms of thiamin, and 86 micrograms of riboflavin per 100 grams fresh weight, and 10.1 per cent dry matter for the experiment. The varieties and strains ranking high in ascorbic acid content were Superlative, 25.2 mg.; Bountiful, 23.6 mg.; strains from the World Fair x Plentiful cross, 24.8 mg.; and those from the Commodore x Superlative cross, 22.9 mg. However, these varieties and strains were also relatively high in dry matter, averaging 11.0 to 13.6 per cent. Logan snap beans were low in both ascorbic acid, 12.9 mg., and in dry matter, 9.0 per cent. Strains from Commodore x Stringless Black Valentine,[1] Commodore x Superlative, Commodore x Plentiful, and U. S. No. 5 sib x S. B. Valentine crosses were considerably above the average in thiamin for the experiment. Superlative was high in thiamin but also had a high percentage of dry matter. Logan, U. S. No. 5 Refugee, and Bountiful were low in thiamin

[1] Hereafter in this paper Stringless Black Valentine is referred to as S. B. Valentine.

TABLE 1

Ascorbic Acid, Thiamin, and Riboflavin Content on Fresh-Weight Basis and Percentage of Dry Matter of 171 Strains and Varieties of Snap Beans Analyzed in Spring of 1943

Variety or cross	Number of samples or strains	Ascorbic acid	Thiamin	Riboflavin	Dry matter
		mg./100 gm.	*μg./100 gm.*	*μg./100 gm.*	*pct.*
Bountiful	1	23.6	57	78	11.4
Brittle Wax	1	19.3	65	84	8.1
S. B. Valentine	3	20.1	71	85	8.6
Tendergreen	2	22.9	63	86	9.2
Superlative	1	25.2	82	95	13.6
U. S. No. 5 Refugee	1	19.1	55	74	8.9
Logan	7	Range 10.1-15.1	43-66	74-93	7.9-11.9
		Mean 12.9	56	81	9.0
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	32	Range 14.2-29.1	52-114	63-103	7.6-14.5
		Mean 19.9	73	80	10.1
Commodore x World Fair	30	Range 11.9-27.5	57-106	80-131	8.9-14.7
		Mean 19.8	73	103	10.9
Commodore x S. B. Valentine	20	Range 14.2-29.1	44-117	78-183	8.3-14.9
		Mean 21.1	84	108	11.3
U. S. No. 5 sib x Brittle Wax	19	Range 12.6-25.2	52-109	63-90	7.7-14.5
		Mean 18.8	73	78	10.3
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	15	Range 15.7-23.8	57-99	72-103	7.4-10.0
		Mean 20.4	72	85	8.4
Commodore x Superlative	11	Range 18.9-29.1	58-112	67-107	9.1-15.5
		Mean 22.9	80	93	11.0
U. S. No. 5 sib x S. B. Valentine	7	Range 19.1-28.2	60-98	74-95	7.9-15.0
		Mean 21.7	78	83	9.8
World Fair x Plentiful	5	Range 21.3-29.4	60-96	90-112	8.8-14.8
		Mean 24.8	73	101	12.4
Commodore x Plentiful	5	Range 13.9-23.0	52-99	93-151	8.9-11.9
		Mean 18.8	78	112	10.2
(Brittle Wax x U. S. No. 5 sib x Brittle Wax) x Tendergreen	4	Range 19.3-20.4	62-84	65-76	8.5-10.4
		Mean 19.8	70	73	9.4
(Brittle Wax x U. S. No. 5 sib x Brittle Wax) x S. B. Valentine	3	Range 15.1-23.6	52-68	67-84	10.4-11.3
		Mean 18.6	59	80	10.7
U. S. No. 5 sib	2	Range 17.2-22.4	65-80	67-86	9.4- 9.4
		Mean 19.8	72	76	9.4
U. S. No. 5 sib x Blue Lake	2	Range 15.2-18.1	74-80	59-63	9.4-10.5
		Mean 16.6	77	61	10.0
Average of strain and variety means	....	20.3	71	86	10.1

content. Strains from Commodore x Plentiful, Commodore x S. B. Valentine, Commodore x World Fair, and World Fair x Plentiful crosses averaged high in riboflavin for the experiment; while those from U. S. No. 5 Refugee, U. S. No. 5 sib, and U. S. No. 5 sib x Blue Lake were low. The dry-matter content of all beans analyzed averaged 9.1 per cent for the first week and 11.0 per cent for the second week.

Fall 1943 and Spring 1944

Snap beans at three different stages of maturity were analyzed for ascorbic acid, riboflavin, thiamin, and dry-matter content in the fall of 1943 and the spring of 1944.

In a preliminary experiment five strains from an early fall planting were harvested twice within eight days. Marketable beans were graded into three lots for each strain—immature, medium, and green-overmature. The green-overmature beans were not in prime condition but would have been considered edible in nearly all cases. The data indicated a difference, significant at the five-per cent level, among the three stages of maturity in both ascorbic acid and thiamin content on a fresh-weight basis. The medium-mature beans had less ascorbic acid than either of the other two lots but this difference disappeared when the data were expressed on a dry basis. Similarly, while the green-overmature beans contained greater amounts of thiamin on a fresh-weight basis than the other two lots, these differences were greatly diminished on a dry basis. Riboflavin was more variable and no trend was observed.

In both fall of 1943 and spring of 1944 16 varieties and strains of snap beans, of which 14 were the same in both seasons, were harvested twice from each of two replicates of a randomized block-yield test. As in the preliminary experiment the beans from each of the strains and varieties were divided according to maturity into three lots for determination of ascorbic acid, thiamin, riboflavin, and dry-matter content. During both seasons snap beans from Replicate 1 were analyzed Monday and Tuesday, and those from Replicate 2 were analyzed Thursday and Friday. This order for harvesting and analyzing was repeated the following week and led to a confounding of replicates with harvest dates.

Means for the three lots from all varieties and strains analyzed are presented on a fresh-weight basis (Table 2). In the fall the medium-mature beans were lower in ascorbic acid content than either the immature or the green-overmature lots. However, in the spring only the green-overmature snap beans were significantly higher in ascorbic acid content. In each season thiamin was significantly higher on a fresh-weight basis in the green-overmature snap beans than in the other lots. In the spring the medium-mature lot averaged significantly more thiamin than the immature and significantly less than the green-overmature beans. Variations in mean riboflavin content were small among the three maturity lots although in the fall the medium-mature beans contained significantly less riboflavin statistically than the other lots. In the fall the dry-matter content of the immature and medium-mature snap beans averaged about the same (9.7 to 9.9 per cent) but that for the green-overmature beans (11.5 per cent) was significantly higher than the values for the other two lots. In the

TABLE 2

Mean Ascorbic Acid, Thiamin, and Riboflavin Content on Fresh-Weight Basis and Dry-Matter Content of Snap Beans at Three Different Stages of Maturity in Two Seasons [1]

Constituent	Fall 1943					Spring 1944				
	Immature	Medium-mature	Green-over-mature	L. S. D.[1] 5%	L. S. D.[1] 1%	Immature	Medium-mature	Green-over-mature	L. S. D.[1] 5%	L. S. D.[1] 1%
Ascorbic acid [2] (mg./100 gm.)	28.7	27.2	28.8	1.3	NS[3]	20.2	20.3	21.9	0.9	1.2
Thiamin [2] (μg./100 gm.)	61	60	72	4	5	80	89	110	6	8
Riboflavin [2] (μg./100 gm.)	84	80	83	2	3	88	89	90	NS[3]	NS[3]
Dry matter [4] (per cent)	9.7	9.9	11.5	0.4	0.5	8.7	10.0	12.2	0.6	0.8

[1] Least difference required for significance between means. [2] Each mean is an average of 64 determinations (four determinations from each of 16 varieties).
[3] F value from variance analysis nonsignificant at that level. [4] Mean is the average of 128 determinations (eight determinations from each of 16 varieties).

spring the values for average dry-matter content of the three maturity lots (8.7, 10.0, and 12.2 per cent) were significantly different from each other.

The average ascorbic acid, thiamin, riboflavin, and dry-matter content of the varieties and strains of snap beans that were analyzed in the fall of 1943 and spring of 1944 are summarized (Table 3). Statistically significant differences between variety means were found for the constituents determined. Of the 14 strains that appeared in both seasons, B1110, a strain of wax beans from a (Brittle Wax x U. S. No. 5 sib) x Brittle Wax cross, was highest in rank for ascorbic acid both in the fall (35.6 mg. per 100 grams, fresh-weight basis) and in the spring (28.2 mg.). The variety Brittle Wax averaged second highest in ascorbic acid for both seasons. The order of ranking for ascorbic acid content was not very consistent from fall to spring for the remaining strains and varieties. However, for thiamin content all but three of the 14 strains and varieties appearing in each season maintained relatively the same ranking. In the fall the mean thiamin content varied from 46 to 82 micrograms and averaged 64 micrograms per 100 grams, fresh-weight basis. In the spring the same varieties and strains ranged from 72 to 127 micrograms and averaged 93 micrograms. In both seasons strains from (S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine, U. S. No. 5 sib x S. B. Valentine, and (Brittle Wax x U. S. No. 5 sib) x Brittle Wax crosses were the highest ranking for thiamin content and Logan the lowest in the experiment. Ranking high in riboflavin were three strains from the Commodore x S. B. Valentine cross, namely, B1274, B1283, and B1272; while ranking low in riboflavin were two strains from the (Brittle Wax x U. S. No. 5 sib) x Brittle Wax cross, B1110, and B1090.

Fall 1944

In the fall of 1944 snap beans from 38 varieties and strains were analyzed for ascorbic acid, thiamin, riboflavin, carotene, and dry-matter content. The beans were harvested between October 6 and October 18 from two replicates of a randomized block-yield test. Determinations were completed from two pickings of one replicate and from one picking of the second replicate. Failure of electric power prevented analysis of another picking from the second replicate.

Variety and strain means based on determinations from three harvests are presented (Table 4). "F" values from variance analyses of the data indicated that varietal and strain differences in ascorbic acid, thiamin, riboflavin, and carotene content were significant at the one-per cent level. Mean ascorbic acid content of varieties and strains ranged from 17.3 to 32.5 mg. per 100 grams fresh weight and averaged 23.7 mg. for the experiment. Masterpiece and one strain from each of the crosses Commodore x S. B. Valentine, U. S. No. 5 sib x S. B. Valentine, Logan x (Commodore x S. B. Valentine), and Fullgreen [2] x (Commodore x S. B. Valentine) were high on a fresh-weight basis in ascorbic acid; while Burpee's Tender

[2] Fullgreen is a variety developed at the Regional Vegetable Breeding Laboratory for resistance to mosaic, mildew, and bacterial blight.

TABLE 3

Mean Ascorbic Acid, Thiamin, Riboflavin, and Dry-Matter Content[1] of Varieties and Strains of Snap Beans Analyzed in Fall of 1943 and Spring of 1944

Variety or cross	Strain No.	Ascorbic acid		Thiamin		Riboflavin		Dry matter	
		Fall 1943	Spring 1944	Fall 1943	Spring 1944	Fall 1943	Spring 1944	Fall 1943	Spring 1944
		mg./100 gm.		*µg./100 gm.*		*µg./100 gm.*		*pct.*	
Brittle Wax	.....	29.6	24.1	63	81	80	85	10.0	8.9
Logan	.....	24.0	20.0	46	78	80	88	9.4	9.4
S. B. Valentine	.....	27.2	19.9	67	127	86	96	11.0	12.2
Tendergreen, Asgrow	.....	25.8	21.4	64	91	85	97	10.7	10.4
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	B1090	25.6	18.3	60	80	74	76	10.7	10.9
	B1110	35.6	28.2	72	113	68	87	9.3	10.4
Commodore x S. B. Valentine	B1272	25.6	19.4	58	90	85	90	9.8	11.1
	B1274	29.0	20.0	52	93	85	102	9.9	10.9
	B1283	22.4	22.7	55	81	83	98	9.7	12.1
Commodore x World Fair	B1266	31.2	18.3	60	72	86	78	10.1	9.3
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1126	25.7	22.2	82	98	82	81	10.1	**10.3**
	B1129	27.8	18.4	82	116	78	101	10.8	**11.1**
U. S. No. 5 sib x S. B. Valentine	B1135	25.6	22.8	78	103	94	91	12.0	10.0
	B1147	26.5	25.8	71	94	74	88	10.3	9.7
Giant Stringless	.....	34.0	.....	65	.....	93	.....	11.6	.....
Sure Crop Wax	.....	34.3	.....	57	.....	82	.....	10.0	.....
Tender Pod	.....	.....	13.3	.....	71	.....	79	.....	**9.4**
U. S. No. 5 sib	B438-10	.....	21.4	.....	101	.....	89	.....	10.8
Mean for each season	.....	28.2	20.8	64	93	82	89	10.3	10.4
L.S.D. 5-per cent level	.....	3.9	2.2	16	19	11	10	1.2	1.8
1-per cent level	.....	5.2	2.9	21	25	14	13	1.6	2.4

[1] The mean value listed for each constituent for each season is an average of four determinations from each of three maturity levels. Each dry-matter value is an average of eight determinations from each of three maturity levels.

TABLE 4

Mean Ascorbic Acid, Thiamin, Riboflavin, and Carotene Content on Fresh-Weight Basis and Percentage of Dry Matter of Snap Beans Analyzed in Fall of 1944

Variety or cross	Strain No.	Ascorbic acid [1]	Thiamin [1]	Riboflavin [1]	Carotene [1]	Dry matter [2]
		mg./100 gm.	*µg./100 gm.*	*µg./100 gm.*	*µg./100 gm.*	*pct.*
Tender Pod	.....	17.3	62	98	386	10.20
S. B. Valentine	.....	26.4	67	95	324	9.69
Tendergreen	.....	25.0	78	89	338	14.27
Burpee's Stringless Green Pod	.....	23.8	61	92	410	11.16
Masterpiece	.....	28.8	70	111	335	9.89
Logan	.....	19.8	59	87	324	11.01
		19.7	72	89	362	10.79
Commodore x S. B. Valentine	B1246	24.7	62	101	320	9.76
	B1247	31.3	62	103	192	9.93
	B1272-1	22.0	79	93	299	14.24
	B1274	22.9	70	111	407	11.44
	B1272-2	24.9	87	123	393	12.34
	B1282	23.9	90	101	440	11.50
	B1282-3	25.1	90	111	400	10.96
	B-1282-2	24.8	69	112	350	14.64
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	B1091	17.7	62	92	335	9.75
	B1093	22.5	61	95	307	11.05
Green	B1186	23.6	55	89	376	10.64
	B1187	24.8	64	87	384	10.01
	B1106	18.3	59	79	268	9.98
	B1107	21.0	52	100	338	9.39
Wax	B1110	25.2	66	77	44	8.55
Commodore x World Fair	B1226	27.1	60	130	308	11.77
	B1230	18.6	65	111	338	10.36
	B1265	24.5	63	102	450	12.05
	B1266	23.0	64	83	303	10.43
U. S. No. 5 sib x S. B. Valentine	B1136	28.8	74	91	367	11.13
	B1147	24.4	81	89	351	10.62
U. S. No. 5 sib x Brittle Wax	B1150	22.4	81	85	356	9.99
	B1151	20.1	75	97	260	11.83
Logan x (Commodore x S. B. Valentine)	B1417	30.2	81	100	405	12.49
	B1416	24.0	68	94	353	11.49
Logan x (Commodore x World Fair)	B1419	18.4	70	111	390	11.47
	B1420	26.4	68	114	349	11.15
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1120	19.3	64	89	271	10.36
World Fair x Plentiful	B1239	26.9	68	100	321	10.20
Fullgreen	.....	19.4	53	96	340	10.01
Fullgreen x (Commodore x S. B. Valentine)	B1389	32.5	89	78	422	14.66
s [3]	.....	3.0	12	9.7	52	1.6
$\bar{x}$	.....	23.7	69	98	348	11.0
C.V. (pct.)	.....	12.6	17.4	9.9	14.9	14.5
L.S.D. 5-per cent level	.....	4.9	20	16	85	2.5
1-per cent level	.....	6.4	26	21	113	3.4

[1] Each mean is an average of three determinations. [2] Each mean is an average of six determinations. [3] s = standard error of a single observation; $\bar{x}$ = mean of all observations; C. V. = coefficient of variability.

Pod and two strains from (Brittle Wax x U. S. No. 5 sib) x Brittle Wax were low.

Thiamin averaged 69 micrograms per 100 grams, fresh-weight basis, for the experiment. Three strains from the Commodore x S. B. Valentine cross and one strain from the Fullgreen x (Commodore x S. B. Valentine) cross had the highest thiamin values, 87 to 90 micrograms, while Fullgreen and three strains from the (Brittle Wax x U. S. No. 5 sib) x Brittle Wax cross were low, having 52 to 59 micrograms. Other snap beans relatively high in thiamin and averaging approximately 78 micrograms per 100 grams fresh weight were Tendergreen and strains from U. S. No. 5 sib x S. B. Valentine and U. S. No. 5 sib x Brittle Wax crosses. These strains were also high in thiamin on a dry-weight basis.

Values for riboflavin held relatively the same rank on both fresh and dry-weight bases. The range of means was 77 to 130 micrograms of riboflavin per 100 grams, fresh-weight basis, and the average for the experiment was 98 micrograms. Some strains significantly higher in riboflavin were from Commodore x S. B. Valentine, Commodore x World Fair, and Logan x (Commodore x World Fair) crosses. Omitting the wax bean, the carotene values ranged from 192 to 450 micrograms per 100 grams fresh weight and averaged 348 micrograms. Strains from Commodore x S. B. Valentine, Commodore x World Fair, Fullgreen x (Commodore x S. B. Valentine), and Logan x (Commodore x S. B. Valentine) crosses were high in carotene, yet two other strains from the Commodore x S. B. Valentine cross along with one strain from (S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine were low. The wax bean was much lower in carotene, 44 micrograms, than any of the green beans. Burpee's Stringless Green Pod, with 410 micrograms, was highest among the commercial varieties in carotene content, and Burpee's Tender Pod was next, with 386 micrograms. The mean carotene content of the other commercial varieties did not differ greatly, ranging from 324 to 338 micrograms.

Spring and Fall 1945

In the spring and fall of 1945 samples for chemical analyses were taken from snap beans planted in a 7 x 7, lattice-square design with four replicates. The two harvesting periods extended from May 21 through June 6 and from October 22 through November 7. Replicates 1 and 2 were harvested on Mondays and Replicates 3 and 4 on Wednesdays. Determinations for ascorbic acid were completed the day of harvest. Data from each replicate were obtained only for ascorbic acid and yield. After sampling for ascorbic acid, the beans from the two harvested replicates for each strain were combined, thoroughly mixed, stored overnight in the refrigerator, and sampled the next day for thiamin, riboflavin, and dry-weight determinations. Twenty-two of the strains and varieties planted in the spring lattice design were included in the fall experiment. The mean vitamin content of the strains analyzed both in spring and in fall are presented on a fresh-weight basis (Table 5). The least significant difference, the standard error of a single observation, the mean of all observations, and the coefficient of variability values given (Table 5) are based on the statistical analysis, as randomized blocks, of the 49 varie-

ties planted in each season. Analysis of the ascorbic acid values as a lattice-square design indicated a small gain in precision through the use of this design. However, means for ascorbic acid content unadjusted for block effects are given (Table 5) so that the data for all vitamins are presented on the same basis. The combination of replicates before sampling prevented consideration of the thiamin and riboflavin values as lattice squares.

The spring of 1945 was an excellent growing season for snap beans. The strains averaged 65 plants per 32-foot row (seeded 75) and yielded, on an acre basis, 11,204 lb. August and September storms cut the average stand in the fall to 34 plants per plot so that the average yield in October was reduced to 1,906 lb. per acre. However, both ascorbic acid content and riboflavin content had about the same values as in the spring. As in the previous two years the mean thiamin content was higher in the spring, 101 micrograms, than in the fall, 73 micrograms per 100 grams, fresh-weight basis.

Varieties and strains differed significantly at the one-per cent level from each other in ascorbic acid, thiamin, and riboflavin content. The ranking of the strains for each vitamin was relatively the same in each season of 1945. A strain from Commodore x Superlative cross, B1254, was highest in ascorbic acid in each season, 31.9 mg. per 100 grams fresh weight in the spring and 26.8 mg. in the fall. Ranking next highest in ascorbic acid content were Giant Stringless, Florida Belle, S. B. Valentine, Tendergreen, and B 1125, whose mean ascorbic acid content ranged from 24.6 to 28.7 mg. per 100 grams fresh weight in the spring and from 23.8 to 25.9 mg. in the fall. In each season Tender Pod, Logan, and B1661 were lowest in ascorbic acid content.

The variety Giant Stringless and three strains from crosses of Logan, namely, B1762, B1419, and B1733, were the four highest in thiamin content when rankings of the strains for both seasons were considered. The spread in the values of these four was somewhat greater in the spring, 110 to 126 micrograms, than in the fall, 78 to 81 micrograms per 100 grams fresh weight. The variety Full Measure was lowest in thiamin in each season, 88 micrograms in the spring and 62 micrograms in the fall. Variations in the riboflavin content of the strains and varieties considered were not great and the average riboflavin content was about the same for each season, 115 micrograms per 100 grams fresh weight in the spring and 112 micrograms in the fall. In both seasons a few strains, B1680, B1716, and B1646, were consistently higher in riboflavin. The average dry-matter content for the experiment in the spring was 11.2 per cent but in the fall was only 8.4 per cent. In the spring there was a consistent increase in percentage of dry matter for the harvest periods, ranging from 9.3 per cent for the first week to 12.8 per cent for the third week; but in the fall the percentage of dry matter remained about the same (8.2 to 8.4 per cent) throughout the harvesting period.

DISCUSSION

Significant differences between strains and varieties were found in ascorbic acid, thiamin, and riboflavin content of snap beans during six growing

TABLE 5

Mean Ascorbic Acid, Thiamin, and Riboflavin Content on Fresh-Weight Basis and Percentage of Dry Matter of Varieties and Strains of Snap Beans Analyzed in Spring and Fall of 1945

Variety or cross	Strain No.	Ascorbic acid [1]		Thiamin [2]		Riboflavin [2]		Dry matter [1]	
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
		mg./100 gm.		*µg./100 gm.*		*µg./100 gm.*		*pct.*	
Full Measure	.....	23.3	21.6	88	62	116	108	10.9	8.0
Giant Stringless	.....	28.7	25.6	110	81	124	123	11.2	8.8
S. B. Valentine	.....	24.6	25.9	107	76	111	109	11.2	7.9
Florida Belle	.....	25.1	25.8	108	69	105	100	12.0	7.9
Tender Pod	.....	17.5	18.7	88	70	113	115	10.1	7.5
Tendergreen	.....	26.3	23.8	91	69	121	110	10.8	8.0
Logan	.....	19.8	19.5	94	73	100	129	10.5	8.3
Commodore x S. B. Valentine	B1716	21.9	23.2	97	66	135	128	11.0	8.7
	B1680	21.6	20.3	94	66	143	126	11.4	8.9
	B1661	19.9	18.8	98	68	109	109	11.5	8.4
Logan x (Commodore x S. B. Valentine)	B1800	20.2	20.0	94	63	108	117	11.1	8.0
	B1762	23.2	22.6	110	78	122	114	12.1	9.3
Commodore x Superlative	B1646	23.9	22.8	106	67	147	120	12.8	8.6
	B1254	31.9	26.8	103	78	134	112	14.2	8.8
Logan x (World Fair x Commodore)	B1419	20.5	20.1	126	78	116	118	12.5	8.3
Logan x (Logan x Tendergreen)	B1746	23.6	19.6	104	74	120	110	11.7	8.3
Commodore x Plentiful	B1270	20.9	23.7	112	74	116	113	11.7	8.5
Commodore x World Fair	B1625	22.6	23.8	116	68	119	111	12.4	7.7
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1125	26.0	25.0	103	76	116	120	11.2	8.6
Miscellaneous crosses with Logan	B1468	20.5	20.4	96	75	98	117	10.9	8.6
	B1733	22.4	20.4	114	78	97	106	11.1	8.6
Fullgreen	B1543	22.0	20.1	104	76	108	104	10.4	8.0
L.S.D.[3] 5 per cent	.....	4.1	4.4	19.0	10.8	10.9	10.2	1.2	0.4
1 per cent	.....	5.5	5.8	25.3	14.4	14.5	13.7	1.6	0.5
s[3]	.....	2.5	2.4	14	13	8.1	5.1	1.0	0.3
$\bar{x}$	.....	23.2	22.9	101	73	115	112	11.2	8.4
C.V. (pct.)	.....	10.7	10.5	13.9	17.8	7.0	4.5	8.9	3.6

[1] Mean of 12 determinations. [2] Mean of six determinations. [3] Based on analysis of group of 49 varieties and strains of which only 22 are included in table. s = standard error of a single observation; $\bar{x}$ = mean of all observations; C. V. = coefficient of variability.

seasons at Charleston, S. C. Data from varieties and strains of snap beans analyzed during three or more seasons are summarized (Table 6). Despite variability in relative rankings for vitamin content from season to season some varieties and strains of snap beans consistently maintained relatively high or low ranks.

Varieties and strains ranking high in ascorbic acid for several seasons and having high mean values were B1110, Giant Stringless, B1247, and B1254. Others, such as Burpee's Tender Pod, Logan, and B1419, were consistently low in ascorbic acid from season to season. Varietal variation in ascorbic acid content of snap beans has been shown by a number of investigators—Heinze *et al.* (1944), Janes (1944), Mack, Tapley, and King (1939), Owens, Anderson, and Smith (1945), and Wade and Kanapaux (1943). Mack, Tapley, and King (1939) found Refugee beans to be low in ascorbic acid in each of three years. Mayfield and Richardson (1939) analyzed Burpee's Stringless Green Pod in two different years and reported an average ascorbic acid content of 19 and 27 milligrams. In the present tests the mean ascorbic acid content for all varieties and strains averaged about the same each season, except in the fall of 1943 when the mean was considerably higher. The fall of 1943 was warm and dry with much above the average percentage of sunshine. Irrigation made growing conditions favorable.

Bedford, McGregor, and Watts (1945) reported no significant varietal difference in the mean ascorbic acid, thiamin, and riboflavin content of Landreth Green Pod, Tendergreen, and S. B. Valentine beans. Fincke, Perkins, and Davey (1944) found the thiamin content of three varieties of snap beans to range from 80 to 124 micrograms per 100 grams, fresh-weight basis. However, only three varieties were tested in each of these reports and the results cannot be considered as indicative of varietal variation. As shown (Table 6) Strains B1419 and B1135 ranked high in thiamin in each season tested. Strain B1254 was high in thiamin as well as in ascorbic acid, and the sister lines, B1125, B1126, and B1129, all ranked high in thiamin in the seasons in which they were analyzed. Mean thiamin content was low in B1090, B1247, Logan, and Brittle Wax. Commercial varieties included (Table 6) were not outstanding in thiamin, although means for S. B. Valentine and Giant Stringless beans were fairly high.

Because of a change in chemical method of analysis for riboflavin in the fall of 1944, care must be taken in comparing the values for riboflavin (Table 6). Strains ranking high in riboflavin were B1226, B1257, B1230, B1419, and B1283. The mean for all seasons for Strain B1274 was not high, but this strain was among the five highest-ranking strains in each season in which it was analyzed. Commodore is in the parentage of all these strains. Giant Stringless averaged high in riboflavin. Comparisons of mean values for spring 1943, fall 1943, and spring 1944 with each other and of spring 1945 with fall 1945 show little difference in average riboflavin content of the snap beans between spring and fall seasons. In Missouri, Hibbard and Flynn (1945) found the fall crop of snap beans to be richer in carotene and riboflavin than the midsummer crop, but the latter crop

TABLE 6

Mean Ascorbic Acid, Thiamin, and Riboflavin Content on Fresh-Weight Basis of 28 Varieties and Strains of Snap Beans Analyzed During Six Growing Seasons

Variety or cross	Strain No.	Ascorbic acid (milligrams per 100 grams fresh weight)						Variety or strain means
		1943		1944		1945		
		Spring	Fall	Spring	Fall	Spring	Fall	
S. B. Valentine	.....	20.1	27.2	19.9	26.4	24.6	25.9	24.0
Brittle Wax	.....	19.3	29.6	24.1	.....	.....	.....	24.3
Giant Stringless	.....	21.6	35.7	.....	.....	28.7	25.6	27.9
Logan	.....	12.9	24.0	20.0	19.6	19.8	19.5	19.3
Tendergreen	.....	22.9	25.8	21.4	25.0	26.3	23.8	24.2
Burpee's Tender Pod	.....	.....	.....	13.3	17.3	17.5	18.7	16.7
Fullgreen	.....	.....	.....	.....	19.4	22.0	20.1	20.5
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	B1090	19.0	25.6	18.3	.....	.....	.....	21.0
	B1110	29.1	35.6	28.2	25.2	.....	.....	29.5
	B1187	18.3	.....	.....	24.8	.....	23.8	22.3
Commodore x Plentiful	B1270	18.2	.....	.....	.....	20.9	23.7	20.9
Commodore x Superlative	B1254	21.0	.....	.....	.....	31.9	26.8	26.6
	B1257	20.1	.....	.....	.....	23.9	22.8	22.3
Commodore x S. B. Valentine	B1247	19.0	.....	.....	31.3	.....	31.2	27.2
	B1272	19.7	25.6	19.4	22.0	.....	.....	21.7
	B1273	21.7	.....	.....	.....	19.9	18.8	20.1
	B1274	20.2	29.0	20.0	22.9	.....	.....	23.0
	B1283	.....	22.4	22.7	.....	21.6	20.3	21.8
Commodore x World Fair	B1226	21.6	.....	.....	27.1	.....	22.2	23.6
	B1230	19.6	.....	.....	18.6	22.6	23.8	21.2
	B1266	24.4	31.2	18.3	23.0	.....	.....	24.2
Logan x (Commodore x S. B. Valentine)	B1416	.....	.....	.....	24.0	20.2	20.0	21.4
Logan x (Commodore x World Fair)	B1419	.....	.....	.....	18.4	20.5	20.1	19.7
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1125	17.7	.....	.....	.....	26.0	25.0	22.9
	B1126	18.8	27.4	22.2	.....	.....	.....	22.8
	B1129	22.7	27.8	18.4	.....	.....	20.1	22.2
U. S. No. 5 sib x S. B. Valentine	B1135	19.5	25.6	22.8	.....	.....	.....	22.6
	B1147	22.0	26.5	25.8	24.4	.....	24.0	24.5
Mean of those listed	.....	20.4	27.9	21.0	23.1	23.1	22.8	22.8

TABLE 6 *(Concluded)*

Mean Ascorbic Acid, Thiamin, and Riboflavin Content on Fresh-Weight Basis of 28 Varieties and Strains of Snap Beans Analyzed During Six Growing Seasons

Variety or cross	Thiamin (micrograms per 100 grams fresh weight)							
	Strain No.	1943		1944		1945		Variety or strain means
		Spring	Fall	Spring	Fall	Spring	Fall	
S. B. Valentine	.....	70	67	127	67	107	76	86
Brittle Wax	.....	65	63	81	....	....	....	70
Giant Stringless	.....	....	65	....	....	110	81	85
Logan	.....	56	46	78	66	94	73	69
Tendergreen	.....	63	64	91	78	91	69	76
Burpee's Tender Pod	.....	....	....	71	62	88	70	73
Fullgreen	.....	....	....	....	53	104	76	78
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	B1090	52	60	80	....	....	....	64
	B1110	62	72	113	66	....	....	78
	B1187	90	....	....	64	....	75	76
Commodore x Plentiful	B1270	52	....	....	....	112	74	79
Commodore x Superlative	B1254	88	....	....	....	103	78	90
	B1257	88	....	....	....	106	67	87
Commodore x S. B. Valentine	B1247	76	....	....	62	....	66	68
	B1272	77	58	90	79	....	....	76
	B1273	110	....	....	....	98	68	92
	B1274	95	52	93	70	....	....	78
	B1283	....	55	81	....	94	66	74
Commodore x World Fair	B1226	68	....	....	60	....	87	72
	B1230	82	....	....	65	116	68	83
	B1266	90	60	72	64	....	....	72
Logan x (Commodore x S. B. Valentine)	B1416	....	....	....	68	94	63	75
Logan x (Commodore x World Fair)	B1419	....	....	....	70	126	78	91
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1125	90	....	....	....	103	76	90
	B1126	74	82	98	....	....	....	85
	B1129	58	82	116	....	....	97	88
U. S. No. 5 sib x S. B. Valentine	B1135	92	78	103	....	....	....	91
	B1147	60	71	94	81	....	87	79
Mean of those listed	.....	75	65	93	67	103	75	79

Variety or cross	Strain No.	Riboflavin (micrograms per 100 grams fresh weight)						Variety or strain means
		1943		1944		1945		
		Spring	Fall	Spring	Fall[1]	Spring	Fall	
S. B. Valentine	.....	85	86	96	95	111	110	97
Brittle Wax	.....	84	80	85	....	....	....	83
Giant Stringless	.....	....	93	....	....	124	123	113
Logan	.....	81	80	88	89	100	129	94
Tendergreen	.....	86	85	97	89	121	110	98
Burpee's Tender Pod	.....	....	....	79	98	113	115	101
Fullgreen	.....	....	....	....	96	108	104	103
(Brittle Wax x U. S. No. 5 sib) x Brittle Wax	B1090	88	74	76	....	....	....	79
	B1110	84	68	87	77	....	....	79
	B1187	76	....	....	87	....	102	88
Commodore x Plentiful	B1270	105	....	....	....	116	113	111
Commodore x Superlative	B1254	84	....	....	....	134	112	110
	B1257	93	....	....	....	147	120	120
Commodore x S. B. Valentine	B1247	96	....	....	103	....	115	105
	B1272	98	85	90	93	....	....	92
	B1273	118	....	....	....	109	109	112
	B1274	106	85	102	111	....	....	101
	B1283	....	83	98	....	143	126	112
Commodore x World Fair	B1226	118	....	....	130	....	123	124
	B1230	131	....	....	111	119	111	118
	B1266	99	86	78	83	....	....	86
Logan x (Commodore x S. B. Valentine)	B1416	....	....	....	94	108	117	106
Logan x (Commodore x World Fair)	B1419	....	....	....	111	116	118	115
(S. B. Valentine x U. S. No. 5 sib) x S. B. Valentine	B1125	76	....	....	....	116	120	104
	B1126	101	82	81	....	....	....	88
	B1129	93	78	101	....	....	122	98
U. S. No. 5 sib x S. B. Valentine	B1135	88	94	91	....	....	....	91
	B1147	74	74	88	89	....	101	85
Mean of those listed	.....	94	82	89	97	119	115	100

[1] Change in method of chemical analysis from that used in preceding seasons.

content of three varieties of snap beans picked and analyzed several times in an eight-day period. The same varieties were also harvested and analyzed for ascorbic acid and riboflavin several times in a 15-day period, but harvest means were variable and no trend was observed.

Varietal variation in carotene content of snap beans was found in the fall of 1944. Janes (1944) reported Tendergreen beans to be consistently higher in carotene on a fresh-weight basis than Bountiful beans in seven different locations in Florida. Owens, Anderson, and Smith (1945) in a study of 11 varieties of snap beans found some varietal variation in carotene content and also noted a tendency for carotene to decrease as the harvesting period progressed. At Charleston in the fall of 1944 three harvests were made at weekly intervals. Mean carotene content was higher and the dry-matter content lower on the first harvesting date than on the second. The first two harvests of the varieties and strains were from the same field replicate but the third harvest was from another replicate. The mean content of carotene and of dry matter for the third harvest was similar to the values for the first harvest.

The vitamin content of marketable snap beans at three different stages of maturity were studied in fall 1943 and spring 1944. On a fresh-weight basis the green-overmature beans averaged significantly more thiamin and slightly more ascorbic acid than the medium-mature or immature beans. This is in line with results of a detailed study by Hibbard and Flynn (1945) on the effect of maturity on the seeds, pods, and whole beans of the Tendergreen variety. Phillips and Fenton (1945) also found overmature Tendergreen beans to be highest in thiamin content on a fresh-weight basis. They reported ascorbic acid and riboflavin content of Tendergreen beans to be greatest, on a fresh-weight basis, in immature beans, although differences owing to maturity were not so great in the case of riboflavin. At Charleston the riboflavin content of the three maturity lots averaged, on a fresh-weight basis, about the same in the spring; but in the fall the value for the medium-mature lot was lower than for the other two groups.

In the fresh market, snap beans are bought on the basis of appearance instead of nutritive value. Green-overmature beans are discriminated against and rejected by customers. Some varieties that become overmature slowly, such as Logan, Tenderpod, and U. S. No. 5, may be of greater nutritional significance than Bountiful or S. B. Valentine which, although ranking relatively high in analyzed constituents, quickly become fibrous and overmature with a consequent loss in consumer appeal. Some progress is being made in the breeding work in combining higher nutritive values with slower rates of fiber development and of overmaturing; such strains as B1147, B1419, B1420, and Fullgreen are examples of this.

Correlation coefficients between the several vitamins and percentage of dry matter were determined using the variety and strain means for each season. Positive correlations significant at the five-per cent level were found between thiamin and ascorbic acid in fall 1944, between thiamin and riboflavin in the spring of 1943 and of 1944, and between thiamin and carotene in fall 1944. The coefficients of correlation did not approach the one-

was higher in ascorbic acid. They reported little difference between summer and fall crops in thiamin content.

The mean ascorbic acid, thiamin, riboflavin, and dry-matter content of all varieties and strains analyzed on each harvesting day during a season are summarized (Table 7). Data from spring 1943 are not included in

TABLE 7

Mean Ascorbic Acid, Thiamin, and Riboflavin Content on a Fresh-Weight Basis and Percentage of Dry Matter of Whole Raw Snap Beans for Each Harvest in Each of Five Crop Seasons

Year	Season	Harvests				L. S. D.[1]	
		1	2	3	4	5%	1%
Ascorbic acid (milligrams per 100 grams)							
1943	Fall	28.3	30.9	26.6	27.1	2.0	2.6
1944	Spring	18.7	20.5	21.3	22.7	1.1	1.4
	Fall	21.9	23.2	26.0	.....	1.4	1.8
1945	Spring	20.5	22.9	26.3	.....	0.5	0.6
	Fall	22.8	21.1	24.9	.....	0.5	0.6
Thiamin (micrograms per 100 grams)							
1943	Fall	55	67	66	70	8	10
1944	Spring	67	84	112	108	9	12
	Fall	73	75	58	.....	6	7
1945	Spring	78	84	140	.....	4	5
	Fall	74	68	77	.....	4	5
Riboflavin (micrograms per 100 grams)							
1943	Fall	75	80	86	88	5	7
1944	Spring	88	90	88	90	NS	NS
	Fall	103	93	97	.....	4	6
1945	Spring	112	115	119	.....	2	3
	Fall	118	111	108	.....	1	2
Dry matter (per cent)							
1943	Fall	9.7	10.3	10.7	10.6	0.6	0.8
1944	Spring	8.8	9.4	11.6	11.9	0.9	1.2
	Fall	10.7	11.9	10.7	.....	0.7	1.0
1945	Spring	9.3	11.4	12.8	.....	1.2	0.4
	Fall	8.4	8.2	8.4	.....	1.6	0.5

[1] Least difference required for significance between means.

the table as different groups of strains and varieties were analyzed each harvesting day. In four seasons mean ascorbic acid content was higher on the last harvesting day of the season than on other harvesting days. Each year snap beans harvested in the spring averaged higher in thiamin content than those grown in the fall. Although thiamin content increased during each season except in the fall of 1944, the increase was greater in the spring as warmer spring weather favors more rapid seed development than the cooler fall weather. Although differences among harvest means for riboflavin content were statistically significant, the range in value of the means was small. There was a greater increase in dry-matter content during the spring harvesting periods than in the fall. Bedford, McGregor, and Watts (1945) reported no significant harvest differences in thiamin

content of three varieties of snap beans picked and analyzed several times in an eight-day period. The same varieties were also harvested and analyzed for ascorbic acid and riboflavin several times in a 15-day period, but harvest means were variable and no trend was observed.

Varietal variation in carotene content of snap beans was found in the fall of 1944. Janes (1944) reported Tendergreen beans to be consistently higher in carotene on a fresh-weight basis than Bountiful beans in seven different locations in Florida. Owens, Anderson, and Smith (1945) in a study of 11 varieties of snap beans found some varietal variation in carotene content and also noted a tendency for carotene to decrease as the harvesting period progressed. At Charleston in the fall of 1944 three harvests were made at weekly intervals. Mean carotene content was higher and the dry-matter content lower on the first harvesting date than on the second. The first two harvests of the varieties and strains were from the same field replicate but the third harvest was from another replicate. The mean content of carotene and of dry matter for the third harvest was similar to the values for the first harvest.

The vitamin content of marketable snap beans at three different stages of maturity were studied in fall 1943 and spring 1944. On a fresh-weight basis the green-overmature beans averaged significantly more thiamin and slightly more ascorbic acid than the medium-mature or immature beans. This is in line with results of a detailed study by Hibbard and Flynn (1945) on the effect of maturity on the seeds, pods, and whole beans of the Tendergreen variety. Phillips and Fenton (1945) also found overmature Tendergreen beans to be highest in thiamin content on a fresh-weight basis. They reported ascorbic acid and riboflavin content of Tendergreen beans to be greatest, on a fresh-weight basis, in immature beans, although differences owing to maturity were not so great in the case of riboflavin. At Charleston the riboflavin content of the three maturity lots averaged, on a fresh-weight basis, about the same in the spring; but in the fall the value for the medium-mature lot was lower than for the other two groups.

In the fresh market, snap beans are bought on the basis of appearance instead of nutritive value. Green-overmature beans are discriminated against and rejected by customers. Some varieties that become overmature slowly, such as Logan, Tenderpod, and U. S. No. 5, may be of greater nutritional significance than Bountiful or S. B. Valentine which, although ranking relatively high in analyzed constituents, quickly become fibrous and overmature with a consequent loss in consumer appeal. Some progress is being made in the breeding work in combining higher nutritive values with slower rates of fiber development and of overmaturing; such strains as B1147, B1419, B1420, and Fullgreen are examples of this.

Correlation coefficients between the several vitamins and percentage of dry matter were determined using the variety and strain means for each season. Positive correlations significant at the five-per cent level were found between thiamin and ascorbic acid in fall 1944, between thiamin and riboflavin in the spring of 1943 and of 1944, and between thiamin and carotene in fall 1944. The coefficients of correlation did not approach the one-

per cent level in any of these instances. Dry-matter content was positively correlated with ascorbic acid at the one-per cent level in the spring of 1943 and of 1944 and at the five-per cent level in the fall of 1945. Dry-matter content was positively correlated with thiamin at the one-per cent level in the fall of 1944 and at the five-per cent level in the spring of 1944 and of 1945. Dry-matter content showed positive correlation with riboflavin at the five-per cent level in four seasons. Correlation between carotene and dry matter was not significant in the fall of 1944, the only test made.

SUMMARY

Over 200 strains and varieties of snap beans were analyzed for ascorbic acid, thiamin, riboflavin, and dry-matter content during six growing seasons at Charleston, South Carolina. The carotene content of 38 strains and varieties of snap beans was determined in one season. Snap beans averaged 22.8 milligrams of ascorbic acid, 79 micrograms of thiamin, 100 micrograms of riboflavin, and 348 micrograms of carotene per 100 grams on a fresh-weight basis.

Differences in mean ascorbic acid, thiamin, riboflavin, and carotene values among the strains and varieties analyzed were found to be statistically significant at the one-per cent level. In general, varieties and strains having low or high vitamin content maintained the same relative ranking in the different seasons although total amounts varied from season to season.

Ascorbic acid content tended to average the same for all seasons except the fall of 1943. In general, the mean ascorbic acid content for all varieties and strains was highest on the last harvesting days of a season. In each of three years the spring crop of snap beans averaged higher in thiamin than the fall crop. There was a greater increase in thiamin and in dry-matter content in the spring seasons than in the fall. Variations in riboflavin content during a season were not great and no consistent trends were observed.

During two seasons, immature, medium-mature, and green-overmature beans of 16 varieties and strains were analyzed for ascorbic acid, thiamin, riboflavin, and dry matter. On a fresh-weight basis significantly more thiamin and slightly more ascorbic acid were found in the green-overmature beans. Riboflavin values ran higher in the spring than in the fall but variations between maturity lots were small. There was a greater difference in dry-matter content of the three maturity lots in the spring than in the fall.

No correlations statistically significant at the one-per cent level were found between ascorbic acid, thiamin, riboflavin, and carotene content of the varieties and strains analyzed although in certain crop seasons a few correlations were significant at the five-per cent level. Dry-matter content was positively correlated with ascorbic acid, with thiamin, and with riboflavin at either the five-per cent or the one-per cent level in several of the crop seasons.

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EFFECT OF CERTAIN HOME PRACTICES ON RIBOFLAVIN CONTENT OF CABBAGE, PEAS, SNAP BEANS, AND SPINACH [1]

FRANCES O. VAN DUYNE, JEAN T. CHASE, ROYENE F. OWEN, AND JANET R. FANSKA

Department of Home Economics, University of Illinois, Urbana, Illinois

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This study was undertaken to determine the effect of certain home practices on the retention of riboflavin in some commonly used vegetables. Three general procedures were employed and the amounts of riboflavin present before and after each treatment were determined. Cabbage was shredded and held in water for one and three hours and in air for two hours; cabbage, peas, snap beans, and spinach were cooked in various amounts of water for different lengths of time; and cabbage was cooked, cooked and held in the refrigerator, and cooked, held, and reheated.

Although information is available in the literature concerning the riboflavin content of raw vegetables, little has been reported on the effect of cooking, holding, and reheating on the retention of this vitamin. Investigations have been carried on to determine the effect of varying the amounts of cooking water and the lengths of cooking time. These indicate that, while riboflavin is not readily destroyed by heat, such variations in procedure influence the distribution of riboflavin between the cooked vegetable and the cooking water. A brief survey of the literature follows.

Cheldelin, Woods, and Williams (1943) studied the effect of steaming on the riboflavin content of cabbage. The raw cabbage contained 57 micrograms of riboflavin per 100 grams and lost 16 per cent during a 30-minute steaming period.

Oser, Melnick, and Oser (1943) observed that raw peas with a riboflavin content of 2.58 micrograms per gram retained 93 and 69 per cent of this amount during cooking in a very small amount of water and in a larger amount of water for a longer period of time, respectively. The peas analyzed by Johnston, Schauer, Rapaport, and Deuel (1943) contained 203 micrograms of riboflavin per 100 grams. When cooked in aluminum pans, 64 per cent was retained; similar results were obtained when Pyrex pans were used for cooking. Mackinney and Sugihara (1942) determined the riboflavin content of raw and cooked peas and found that the values obtained before and after cooking were in the same range. Little difference in riboflavin content was noted by Rose and Phipard (1937) for raw peas and peas cooked in water to barely cover for 15 minutes.

Phillips and Fenton (1945) reported an average value of 0.140 milligram of riboflavin per 100 grams of raw Tendergreen snap beans; 78 per

[1] Work undertaken as part of the National Cooperative Experiment Station Project on "Conservation of Nutritive Values of Foods."

cent was retained in the cooked vegetable and 18 per cent was present in the cooking water. Farrell and Fellers (1942) found that there was no apparent loss of riboflavin when freshly harvested Bountiful snap beans, containing 132 micrograms of riboflavin per 100 grams, were cooked in one-fourth their weight of water for 30 minutes.

A variation from 0.174 to 0.245 milligram of riboflavin per 100 grams in garden-fresh spinach was observed by Gleim, Tressler, and Fenton (1944). When 300 grams were cooked in twice that weight of water for a total of eight minutes, the vegetable retained 52 per cent of the riboflavin with 36 per cent in the cooking water. Decreasing to 60 grams the amount of water used increased the retention of riboflavin in the cooked spinach to 75 per cent. Phillips, Dickerson, and Fenton (1943) analyzed spinach purchased on the market and found from 0.202 to 0.257 milligram of riboflavin per 100 grams. Cooked in an approximately equal weight of water for six minutes, the spinach retained 68 per cent of the original riboflavin content, while the cooking water contained 36 per cent. When cooked in water clinging to the leaves for 12 minutes, 83 per cent was retained; not enough cooking water remained for analysis. Cheldelin, Woods, and Williams (1943) found that market spinach contained 220 micrograms of riboflavin per 100 grams. Steaming resulted in a 27-per cent loss of riboflavin.

EXPERIMENTAL PROCEDURE

The Resistant Detroit cabbage, Black Valentine snap beans, and Virginia Savoy (Blight Resistant) spinach used in this study were provided by the Department of Horticulture of the University of Illinois. The snap beans and spinach were harvested at suitable stages of maturity and brought to the food-research laboratory on the day they were to be used. Snap beans were obtained from August 10 to September 28, 1944, and spinach from October 9 to 27 the same year. Cabbage was harvested on October 11, 1944, and placed in 50-pound, tightly covered lard cans. These were stored at the Horticulture Field Laboratory where the temperature varied from 0.56 to 3.33°C.(33 to 38°F.). From November 8, 1944, until January 10, 1945, one can of cabbage at a time was brought to the laboratory for sampling and held in a refrigerator at 4 to 6°C.(39.2 to 42.8°F.) until completely used. The cabbage employed to determine the effect of cooking, holding, and reheating on the riboflavin content was of an unknown variety purchased on the local market from January 16 to February 22, 1945. Peas, purchased on the local market over an extended period of time, were also of an unknown variety. Both of these vegetables were obtained the day before they were to be tested, and stored overnight in a refrigerator at 4 to 6°C.

In preparation for sampling, the heads of cabbage were washed, outer leaves were removed, and the cabbage was dried with a towel. Peas were shelled, washed twice in cold water, and dried on towels. Snap beans were washed twice in cold water, dried on towels, the ends were snapped, and the beans were cut into 1½-inch pieces. After the tough portions of the stems had been discarded, spinach was washed three times and allowed to drain in dishpans for several minutes before the samples were weighed. Moisture determinations were made on all raw vegetables.

The three general procedures which follow are commonly practiced in the home and have been investigated to determine their effect on the riboflavin content of these vegetables.

Holding in Water or in Air for Different Lengths of Time: Four heads of Resistant Detroit cabbage were cut into quarters after preparation for sampling. Lots for treatment were made up of one quarter from each head. A stainless-steel knife was used to shred the cabbage in ⅛-inch slices and these were well mixed. A 300-gram sample of shredded cabbage was taken from each lot; the first was allowed to remain one hour in one liter of tap water (water to cover), another in air for two hours, and the third in one liter of water for three hours. The four remaining quarters were shredded as before, mixed and sampled at once for riboflavin determinanations. After completion of the treatment the two lots which had been held in water were drained in enamel colanders and dried on towels before weighing and sampling. Each experiment was repeated five times.

Varying Amounts of Water Used and Times of Boiling: Four hundred-gram samples of Resistant Detroit cabbage, taken from quarters of four heads, cut in ¾-inch slices, and 300-gram samples of Black Valentine snap beans, cut in 1½-inch pieces, were boiled in one-half and twice their weights of water for the shortest time required to give a tender product, 10 and 15 minutes, respectively. Samples were also boiled in double their weights of water for twice the minimum boiling time. Three hundred-gram samples of peas were boiled in an equal weight of water or two times as much water for the minimum time required to give a tender "done" product and in twice their weight of water for double the minimum boiling time. Four hundred-gram portions of Virginia Savoy (Blight Resistant) spinach were cooked in water clinging to the leaves after washing, and in half and twice their weight of water for seven minutes. In this instance, seven minutes was the total cooking time, including that required for boiling to be resumed after the vegetable was added to the water; lengths of the boiling periods are given (Table 2). The vegetables were cooked in two-quart aluminum pans with fitted covers containing a steam vent. Details of the cooking procedure—regulation of gas burners, control of amounts of water and salt used, and draining and weighing of the cooked products—were carried out according to the mimeographed outline of Recommended Laboratory Procedures for Food Preparation and Food Preservation for the National Cooperative Experiment Station Project on "Conservation of Nutritive Value of Foods." In the case of spinach these were modified in that 1.5 grams of salt were added to the spinach cooked in water clinging to the leaves and all lots of cooked spinach were drained in enamel colanders for three minutes, being turned with a fork during the process. The experiment was repeated four times for each vegetable.

Cooking, Holding, and Reheating: Cabbage purchased on the local market was sampled and cooked as in the previous experiment, three lots of 400 grams each in 800 milliliters of water for 10 minutes. The remaining lot of raw cabbage, one lot of the cooked and all the cooking waters were sampled immediately for riboflavin determinations, while the other two lots were allowed to cool for one hour then held in the refrigerator for

48 hours. One of these was sampled without further treatment while the other was reheated by boiling in 100 milliliters of water for two minutes, then drained and sampled. The water used in reheating the cabbage was also analyzed. Each experiment was repeated five times.

The formulation of a method for the determination of riboflavin was influenced by the reports of Andrews (1943, 1944) and of Peterson, Brady, and Shaw (1943) and by preliminary work in this laboratory.

Preparation of Extracts: Samples of vegetables (60 grams of peas or spinach and 75 grams of cabbage or snap beans) were ground in a Waring blendor with 200 milliliters of 0.1 N H_2SO_4—spinach for six minutes, cabbage and peas for eight minutes, and snap beans for 10 minutes. The mixtures were quantitatively transferred to volumetric flasks; the spinach extract was diluted to one liter and the others to 500 milliliters with 0.1 N H_2SO_4. Ten- to 30-milliliter aliquots of these extracts and one- to 10-milliliter portions of undiluted cooking water were pipetted into amber glass flasks. Three different dilutions were used except in the case of a few cooking waters; 0.1 N H_2SO_4 was added so that approximately 50 milliliters of it would be present. The samples were autoclaved at 15 pounds pressure for 15 minutes and then cooled. Twelve milliliters of 6.5 per cent $Na_3PO_4 \cdot 12H_2O$ and two milliliters of glacial CH_3COOH were added; the mixtures were filtered through No. 42 Whatman filter paper into 100-milliliter volumetric flasks, and made up to volume with water.

Fluorometric Determinations: A Klett fluorimeter with photocell filters 351 and lamp filters 5113 was used. Three aliquots of each filtrate were treated in the following ways and read against a solution of crystalline riboflavin containing 0.1 microgram per milliliter.

Reading	Composition of solution read
A	15 milliliters of filtrate and 15 milliliters of water.
B	15 milliliters of filtrate and 15 milliliters of riboflavin solution (0.05 microgram riboflavin per milliliter).
C	15 milliliters of filtrate, 14.5 milliliters of water, and 0.5 milliliter of $Na_2S_2O_4$ solution (one gram $Na_2S_2O_4$ in 20 milliliters of ice-cold, five-per cent $NaHCO_3$ solution).

Blanks were run on the reagents and corrections made. Calculations were made according to the following formula:

$$\frac{A-C}{B-A}(0.025) = \text{micrograms of riboflavin per milliliter.}$$

RESULTS AND DISCUSSION

Effect of Holding in Water or in Air for Different Lengths of Time: The mean values for the riboflavin content of raw shredded cabbage sampled at once and after holding in water for one and three hours and in air for two hours are presented (Table 1). The results are expressed on the "as determined" and on the equivalent raw-weight bases. Comparing

these latter figures, it is evident that, while slight losses may occur as a result of all treatments, the differences in riboflavin content obtained in these experiments were not statistically significant.[2] The mean value for the riboflavin content of the untreated, shredded Resistant Detroit cabbage was 0.047 ± .0017 milligram per 100 grams. This figure is slightly higher than the 0.038 milligram per 100 grams reported by Munsell (1942) or the range, 0.017 to 0.044 milligram per 100 grams, given by Poole, Heinze, Welch, and Grimball (1944) for seven varieties of raw cabbage. However, the figures reported (Table 1) are between the values of 0.044 and 0.066 milligram per 100 grams obtained by Hodson (1940) and lower than the value of 0.057 milligram per 100 grams determined by Cheldelin, Woods, and Williams (1943). Wood, Collings, Stodola, Burgoin, and Fenton (1946) held freshly shredded cabbage containing 0.032 to 0.047 milligram of riboflavin per 100 grams in air at room temperature for two hours and found no appreciable loss of riboflavin.

TABLE 1

Riboflavin Content of Raw Cabbage[1] After Holding in Water or in Air for Different Lengths of Time

Moisture mean and standard deviation of mean	Treatment of cabbage	Number of determinations	Riboflavin mean and standard deviation of mean[2]		Difference between means[3] and standard error of difference[4]
			As determined	Raw-weight basis	
pct.			*mg./100 gm.*	*mg./100 gm.*	
94.6±.11	Shredded	12	.048±.0017	.048±.0017	.....
	Shredded, held one hour in water	12[5]	.043±.0009	.045±.0011	.003±.0020
94.4±.12	Shredded	12	.047±.0018	.047±.0018	.....
	Shredded, held three hours in water	12[5]	.042±.0018	.045±.0019	.002±.0026
94.3±.13	Shredded	15	.049±.0019	.049±.0019	.....
	Shredded, held two hours in air	15	.047±.0019	.046±.0018	.003±.0026

[1] Resistant Detroit Variety.

[2] Standard deviation of the mean $S\bar{x} = \sqrt{\frac{\Sigma x^2}{n(n-1)}}$.

[3] Difference between the means calculated from values of raw shredded cabbage and treated cabbage on the raw-weight basis.

[4] Standard error of the difference $= \sqrt{(S\bar{x}_1)^2 + (S\bar{x}_2)^2}$.

[5] This experiment was repeated five times but one of the average values of three figures was not included. The criterion applied for rejection is discussed in Fales, H. A., 1925. Inorganic Quantitative Analysis. The Century Company, New York, N. Y.

Effect of Varying Amounts of Water Used and Times of Boiling: Figures for the riboflavin content of cabbage, peas, snap beans, and spinach in the raw state and after being cooked in different amounts of water for various periods of time are presented (Table 2). The mean values are expressed in milligrams of riboflavin per 100 grams of vegetable as deter-

[2] A difference is considered significant if the difference between the means is three or more times the standard error of that difference.

mined, while the percentages are calculated on the basis of the equivalent raw weights and represent the distribution of the riboflavin originally present.

The Resistant Detroit cabbage used in this series of experiments contained 0.056 ± .0017 milligram of riboflavin per 100 grams. Comparison of this figure with that obtained previously (Table 1) offers further evidence of the variability of riboflavin content in different heads of cabbage. Four hundred grams of this cabbage boiled for 10 minutes in 200 milliliters of water retained 74 per cent of the riboflavin present in the raw while 20 per cent was found in the cooking water. After boiling cabbage in twice its weight of water for the same period of time, a significantly larger amount of riboflavin was dissolved in the cooking water and the cooked vegetable retained 50 per cent. Doubling the boiling time resulted in a further slight decrease of riboflavin in the cooked product but this decrease is not significant. As might be expected, these percentage retentions are lower than the 84 per cent obtained by Cheldelin, Woods, and Williams (1943) for cabbage which had been steamed for 30 minutes.

When 300-gram lots of peas which had been purchased on the market were boiled in 300 milliliters of water for 20 minutes, 75 per cent of the original riboflavin content remained in the cooked vegetable and 23 per cent went into solution. Boiling peas in twice their weight of water resulted in a retention of 61 per cent which was significantly less than that obtained with the smaller volume of water. Doubling the cooking time with the larger volume of cooking water did not significantly change the amount of riboflavin present. The 61-per cent retention reported above is comparable to that obtained by Johnston, Schauer, Rapaport, and Deuel (1943) who cooked 85 grams of peas in 180 milliliters of water for 17 minutes; the peas cooked in aluminum pans retained 64 per cent of the original riboflavin content, 2.5 per cent was lost, and the remainder was present in the cooking water. The 75-per cent retention of riboflavin in peas cooked in an equal weight of water is slightly higher than the 69 per cent found by Oser, Melnick, and Oser (1943) when 454 grams of peas were cooked in 360 milliliters of water for 20 minutes. However, all of the figures are lower than the 93-per cent retention reported by these workers for 454 grams of peas cooked in 15 milliliters of water for 15 minutes.

Black Valentine snap beans retained 88, 79, and 73 per cent of the riboflavin present in the fresh raw sample when boiled in half their weight of water for 15 minutes, twice their weight of water for 15 minutes, and twice their weight of water for 30 minutes, respectively. In each case, the remaining percentage was dissolved in the cooking water. These figures show that significantly greater amounts of riboflavin went into solution with an increase in amount of water used and an increase in cooking time. Phillips and Fenton (1945) report that Tendergreen snap beans retained 78 per cent of the riboflavin content when 255 grams of the vegetable were cooked in 250 grams of water for 25 minutes. Although the cooking procedures differ slightly, the results are similar. Farrell and Fellers (1942) found no loss of riboflavin from Bountiful snap beans cooked in one-fourth their weight of water for 30 minutes; the cooking waters are included in the analyses.

TABLE 2

Effect of Boiling in Various Amounts of Water for Different Lengths of Time on Riboflavin Content of Vegetables

Vegetable and variety	Raw		Details of cooking procedure			Number of determinations	Riboflavin content			
							In cooked vegetable—mean and standard deviation of mean as determined	Calculated on raw-weight basis		
	Moisture mean and standard deviation of mean	Riboflavin mean and standard deviation of mean	Weight of vegetables taken for cooking	Amount of water used	Length of boiling period			Retained in the cooked vegetable	Retained in the cooking water	Lost
	pct.	*mg./100 gm.*	*gm.*	*gm.*	*min.*		*mg./100 gm.*	*pct.*	*pct.*	*pct.*
Resistant Detroit cabbage	94.2±.21	.058±.0022	400	200	10	12	.050±.0014	74	20	6
	94.4±.17	.055±.0014	400	800	10	12	.030±.0010	50	44	6
	94.2±.20	.055±.0025	400	800	20	12	.028±.0013	47	49	4
Peas	79.2±.16	.175±.0023	300	300	20	14	.138±.0021	75	23	2
	79.4±.25	.183±.0023	300	600	20	14	.114±.0021	61	40	+1[1]
	79.7±.20	.180±.0035	300	600	40	14	.115±.0018	62	41	+3[1]
Black Valentine snap beans	92.5±.19	.116±.0024	300	150	15	12	.120±.0030	88	12	....
	92.4±.13	.125±.0042	300	600	15	12	.104±.0036	79	23	+2[1]
	92.8±.16	.124±.0031	300	600	30	12	.096±.0028	73	28	+1[1]
Virginia Savoy (Blight Resistant) spinach	91.3±.43	.287±.0107	400	.....[2]	7[3]	12	.318±.0134	69	16	15
	92.2±.41	.265±.0085	400	200	7[3]	12	.224±.0085	50	33	17
	92.0±.34	.262±.0040	400	800	7[3]	12	.157±.0080	36	50	14

[1] Apparent gains are within the experimental error of the method. [2] Water clinging to leaves after washing. [3] Total cooking time.

Virginia Savoy (Blight Resistant) spinach, which contained from 0.262 to 0.287 milligram of riboflavin per 100 grams when sampled within two hours of harvesting, retained 69, 50, and 36 per cent of the nutrient when 400 grams were cooked for seven minutes in water clinging to the leaves after washing, in 200 milliliters of water, and in 800 milliliters of water, respectively. Increasing the volume of cooking water caused significant decreases in the amounts of riboflavin retained in the cooked vegetable. The 69-per cent retention of riboflavin in spinach cooked in water clinging to the leaves is comparable with the 73-per cent retention obtained by Cheldelin, Woods, and Williams (1943) in spinach which was steamed for 10 minutes. However, the percentage retentions obtained in this study

TABLE 3

Effect of Cooking, Holding, and Reheating on Riboflavin Content of Cabbage[1]

Treatment	Moisture mean and standard deviation of mean	Riboflavin content mean and standard deviation of mean as determined	Retention of riboflavin calculated on raw-weight basis	
			In cooked cabbage	In cooking water
	pct.	*mg./ 100 gm.*	*pct.*	*pct.*
Cabbage, raw	93.1±.23	.052±.0020	....	....
Cabbage, cooked (400 gm. in 800 ml. water for 10 minutes)	.....	.029±.0011	51	46
Cabbage, cooked as above, cooled one hour, held 48 hours in refrigerator	.....	.028±.0008	47	46
Cabbage, cooked as above, cooled one hour, held 48 hours in refrigerator, reheated (100 ml. water, boiled two minutes)	.....	.027±.0012	42	48 9

[1] Purchased on the market.

are lower than the majority reported in the literature. When Gleim, Tressler, and Fenton (1944) cooked 300 grams of garden-fresh spinach (Heavy-Pack variety) in 60 milliliters of water, 75 per cent of the riboflavin was retained. Cooking a sample of the same size in twice its weight of water resulted in a 52-per cent retention as compared with 36 per cent in the present study. Fresh market spinach which was analyzed by Phillips, Dickerson, and Fenton (1943) was found to contain 83 per cent of its original riboflavin content after cooking for 12 minutes in water clinging to the leaves and 68 per cent when 600 grams of spinach were cooked in 625 grams of water for six minutes. The variation in the percentage retentions obtained in several investigations may be due to differences in the variety and maturity of the spinach used.

It is interesting to note that, while there was no significant destruction of riboflavin when cabbage, peas, and snap beans were boiled (Table 2), varying percentages were dissolved in the cooking waters. When spinach was cooked, however, the loss ranged from 14 to 17 per cent. In view of the stability of riboflavin, the question arises as to whether the loss is

real or whether all the riboflavin present was determined by the method employed.

Effect of Cooking, Holding, and Reheating: The results obtained when cabbage which had been purchased on the market was cooked, held, and reheated are presented (Table 3). Cabbage boiled in twice its weight of water for 10 minutes retained 51 per cent of the amount of riboflavin present in the raw, a value similar to the 50-per cent retention obtained in the previous experiments with Resistant Detroit cabbage. There was only a slight, insignificant loss of riboflavin as 46 per cent remained in the cooking water. Cabbage which had been cooked, cooled, and held in the refrigerator for 48 hours contained 47 per cent of the original riboflavin, while that which was also reheated retained 42 per cent. The former value is not significantly different from the value for the riboflavin content of cabbage immediately after cooking, while the latter one is.

SUMMARY

The mean values for the riboflavin contents of lots of raw, freshly shredded Resistant Detroit cabbage were 0.048 ± .0017, 0.047 ± .0018, and 0.049 ± .0019 milligram per 100 grams and for comparable lots held one and three hours in water and two hours in air were 0.045 ± .0011, 0.045 ± .0019, and 0.046 ± .0018 milligram per 100 grams, respectively. The differences were not statistically significant.

Resistant Detroit cabbage, peas, Black Valentine snap beans, and Virginia Savoy (Blight Resistant) spinach, when cooked in the smallest volumes of water for the minimum boiling times, contained 0.050 ± .0014, 0.138 ± .0021, 0.120 ± .0030, and 0.318 ± .0134 milligram of riboflavin per 100 grams, respectively. These amounts represent retentions in the cooked vegetables of 74, 75, 88, and 69 per cent. When these vegetables were cooked in twice their weights of water for the same cooking times, the percentage retentions of riboflavin in the cooked vegetables were significantly less than when they were cooked in one-half or equal their weight of water. Doubling the boiling times for cabbage and peas did not cause any further significant reductions in the amounts of nutrient present in the cooked vegetables. In the case of snap beans, however, a significantly larger percentage of riboflavin was found in the cooking water when the boiling time was doubled.

The amounts of riboflavin present in raw cabbage purchased on the market, in cabbage after cooking, after cooking and holding, and after cooking, holding, and reheating were 0.052 ± .0020, 0.029 ± .0011, 0.028 ± .008, and 0.027 ± .0012 milligram per 100 grams, respectively. Cabbage which had been cooked, held, and reheated retained a significantly lower percentage of riboflavin than that which was freshly cooked.

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CONDITION OF ORANGES AS AFFECTING BACTERIAL CONTENT OF FROZEN JUICE WITH EMPHASIS ON COLIFORM ORGANISMS

E. R. WOLFORD AND J. A. BERRY
Western Regional Research Laboratory,[1] Albany, California

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The recent rise in commercial importance of frozen orange juice has stimulated interest in its microbiology, since the product is in no way heat-treated before being frozen. Coliform organisms were detected in orange juice as early as 1929 by Stone (1945) in Los Angeles. Frozen orange juice has not, however, a general reputation as a harborer of *Escherichia coli*, and qualitative bacteriological tests have not commonly been made on the product. Also, the fact that the acid reaction pH 3.5 to 3.6 of the product prevents growth of most bacteria has probably tended to divert attention from its role as a mechanical carrier.

In the broad sense, the microbiology of frozen orange juice should include such considerations as where and in what numbers microorganisms gain entrance to the products, the types of microorganisms represented, the practical means that might be taken to lessen contamination, and, eventually, the microbial content that might be regarded as consistent with sound sanitary practices in a commercial plant. In regard to coliform organisms in orange juice, the opinion has been expressed that they may be associated with bacterial "soft rot" in the fruit used and do not fall in the *Escherichia coli* section of the group. The particular work described here, which is part of a broader investigation in progress, was undertaken to investigate the influence of "soft rot" fruit on numbers and types of bacteria, especially coliforms in orange juice, and to trace the fate of the organisms during prolonged freezing storage of the product. Manuscripts covering other phases of the investigation of the bacteriology of frozen orange juice are now being prepared.

EXPERIMENTAL PROCEDURE

The oranges used in this experiment were California Valencia oranges of processing grade and were taken from the sorting belt in a plant engaged in commercial juicing operations. Two lots of juice were used. One lot, No. 30, was extracted from Valencia oranges showing no visible spoilage; the other lot, No. 31, was from fruit having soft decayed spots varying from about one inch in diameter to over half the surface of the orange. Visible mold growth was not, however, present on the fruit used in this experiment. The oranges in each lot were handled separately, but both lots received the same treatment. The oranges were first washed in a 1:250 solution of a quaternary ammonium compound, removed from the bath,

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

and drained. All equipment used was sterilized and the washed fruit was handled with clean rubber gloves to minimize danger of hand contamination. After draining, the fruit was halved and burred in an electrically driven orange juicer. The juice was strained through a 20-mesh, stainless-steel screen, the first 200 c.c. being discarded. After a sample had been removed for the original analysis, the juice was put in storage at 0°C. (32°F.) until morning (about 15 hours); then it was sealed in small enameled cans and frozen at —17.8°C.(0°F.). At monthly intervals one can of each lot of juice was removed from 0°F. storage, thawed in warm running water, shaken, and aseptically opened. An interesting observation was that the "soft rot" juice smelled "sour," but its pH was 3.74, while that of the "good" juice was 3.64.

A 100-c.c. sample was removed and neutralized with sterile NaOH solution to pH 6.6 to 7 and analyzed for total plate count on Bacto tryptone-glucose-extract agar, pH 7, for yeast and mold count on several types of standard and differential media.

Tryptone-glucose agar was used for total counts because it is richer than nutrient agar and is more likely to give a higher colony count. Potato-dextrose agar was included for yeast and mold counts, since it is in use in laboratories engaged in work with citrus products. Stevens (1945) of the California Fruit Growers' Exchange has found that the organisms which will grow on this medium will also grow on orange juice. Consequently, he regards potato-dextrose agar as one of the most valuable media for control work in citrus processing. Throughout this investigation potato-dextrose agar gave counts which usually ran about 12 per cent lower than the counts on tryptone-glucose-extract agar. In the qualitative tests for coliform organisms, inoculations of 10 c.c., 1 c.c., and 0.1 c.c. were made in standard lactose broth. In the original analyses both standard lactose broth and formate ricinoleate broth were used and dilutions as high as 1:2,000 were made and inoculated into the medium. Material from positive tubes was streaked on eosin-methylene-blue agar and colonies were fished to agar slants and lactose broth for completion of the coliform tests and for further study; results of this investigation are presented (Tables 1 and 2).

It is apparent that the condition of the oranges had a decided influence on the plate counts and coliform index of the frozen juice. The rate of microbial destruction during 0°F. storage was greater in juice No. 31 than in juice No. 30, resulting in a "kill" of approximately 97 per cent as against about 80 per cent over the seven-month period. It should be noted, however, that even at the end of the period mentioned, the juice from the poor oranges still contained manyfold more organisms than were originally present in juice from the sound fruit. Also, after the initial analysis no positive completed coliforms were at any time isolated from sound fruit juice (No. 30), while coliform tests were completed as positives even after eight months of freezing storage in juice from poor fruit (No. 31).

In identification of coliforms, preference was given to Barrit's (1936) modification of the Voges-Proskauer test because of its quick and clear-cut reactions. The methyl red, indole, sodium citrate, uric acid, and Eijkman tests were performed according to standard methods of the Society of

American Bacteriologists (1936). The Vaughn-Levine (1936) boric acid test was found very useful and was run as described. Since the test for hydrogen sulfide, using lead-acetate paper suspended over proteose-peptone agar slants, appeared to be too sensitive to give specific differentiation [the undesirability of very sensitive tests for H_2S in differentiating coliforms has been pointed out by Vaughn and Levine (1942)], bacto-iron-peptone agar was given preference.

TABLE 1

Effect of Storage for Seven Months at —17.8°C.(0°F.) on Bacteria in Juice (No. 30) From "Good" Oranges

Months' storage	Total count per c.c.	Yeast and mold count per c.c.	Coliforms presumptive	Coliforms confirmed	Coliforms completed
0	12,300	10,900	1/c.c.	1/c.c.	1/c.c.
1	8,000	7,800	4/100 c.c.	0/100 c.c.	0/100 c.c.
2	5,400	5,200	0/100 c.c.	0/100 c.c.	0/100 c.c.
4	3,500	2,800	4/100 c.c.	2/100 c.c.	0/100 c.c.
6	1,600	1,050	4/100 c.c.	0/100 c.c.	0/100 c.c.
7	2,800	2,600	2/100 c.c.	0/100 c.c.	0/100 c.c.

TABLE 2

Effect of Storage for Eight Months at —17.8°C.(0°F.) on Bacteria in Juice (No. 31) From "Soft-Rot" Oranges

Months' storage	Total count per c.c.	Yeast and mold count per c.c.	Coliforms presumptive	Coliforms confirmed	Coliforms completed
0	31,500,000	26,500,000	2,000/c.c.	2,000/c.c.	2,000/c.c.
1	3,192,000	3,400,000	3,500/100 c.c.	2,400/100 c.c.	2/100 c.c.
2	3,100,000	2,800,000	1,600/100 c.c.	19/100 c.c.	.....
4	2,300,000	1,980,000	1,600/100 c.c.	36/100 c.c.	17/100 c.c.
6	800,000	960,000	1,600/100 c.c.	14/100 c.c.	14/100 c.c.
7	900,000	605,000	1,600/100 c.c.	23/100 c.c.	23/100 c.c.
8	1,250,000	990,000	1,600/100 c.c.	23/100 c.c.	23/100 c.c.

The cultural characteristics of 12 coliforms isolated from the first analysis of juice No. 31 are given (Table 3). The data make it apparent that these organisms fit the genus *Aerobacter* more closely than *Escherichia*. They are gram-negative organisms which are methyl-red negative and produce acetyl-methyl carbinol; they do not produce acid and gas in the modified Eijkman medium at 46°C.(114.8°F.) or in the Vaughn-Levine boric acid medium at 44°C.(111.2°F.), but they do attack celloboise. The indole test, which is erratic in the series, is not considered reliable for general differentiation.

Three coliform cultures isolated from the fresh juice from good oranges (No. 30) were also characteristically *Aerobacter*, while two other organisms isolated from lactose fermentation tubes showing gas were yeasts. Presumably, they had attacked the sugar in the orange-juice inoculum.

Of the 34 cultures isolated from the "poor" juice during the eight-month storage period, 31 were considered to be *Aerobacter*, since they were characteristic of this genus in their Voges-Proskauer and methyl-red reactions. No further work was done on those 31 cultures, but the three

TABLE 3

Cultural Characteristics of Organisms From "Soft Rot" Orange Juice (No. 31)[1]

No.	E.M.B. agar	Methyl red	Voges-Proskauer	Growth in sodium citrate	Growth in uric acid	Eijkman reaction, 46°C.	Vaughn-Levine boric acid	Nitrates produced	H_2S produced	Indole	Lactose	Starch	Dextrin	Glycerol	Inositol	Salicin	Gelatin liquefaction	Inulin
56	1[2]	—	+	+	+	—	—	+	+	+	AG	—	A	AG	—	—	—	—
57	1	—	+	+	+	—	—	+	—	+	AG	—	A	AG	AG	AG	Slow	—
59	1	—	+	+	+	—	—	+	—	+	AG	AG	A	AG	AG	AG	Slow	—
60	2[3]	—	+	+	+	—	—	+	—	+	AG	AG	AG	AG	AG	AG	Slow	—
61	2	—	+	+	+	—	—	+	—	—	AG	AG	AG	AG	AG	AG	—	AG[4]
62	2	—	+	+	+	—	—	+	—	+	AG	AG	AG	—	AG	AG	Slow	AG
63	1	—	+	+	+	—	—	+	—	—	AG	AG	AG	AG	AG	AG	—	AG
64	2	—	+	+	+	—	—	+	—	+	AG	AG	A	AG	—	—	Slow	—
65	2	—	+	+	+	—	—	+	—	?	AG	—	—	AG	—	AG	—	AG
66	2	—	+	+	+	—	—	+	—	—	AG	AG	AG	AG	AG	AG	—	—
67	2	—	+	+	+	—	—	+	—	—	AG	AG	AG	AG	AG	AG	—	AG
68	2	—	+	+	+	—	—	+	—	—	AG	AG	AG	AG	AG	AG	—	AG

[1] All 12 organisms were gram-negative rods, producing circular, convex colonies with smooth surface and entire margins on agar and a glistening white, filiform-to-spreading growth on agar slants. All were facultative and fermented mannitol, arabinose, rhamnose, dextrose, levulose, sucrose, maltose, galactose, and cellobiose with acid and gas. [2] Type 1 growth shows metallic sheen or dark colonies. [3] Type 2 growth shows viscid, coalescing growth with only slight sheen. [4] Inulin fermentation anaerobic.

which were methyl-red positive and Voges-Proskauer negative were carried through further differentiation tests. Each of these three cultures was from a 10-c.c. portion of the juice, one each from samples at the end of one, four, and six months' storage. The cultures isolated after one and four months were characteristically *Escherichia freundii,* since they did not produce gas in the Eijkman or Vaughn-Levine tests, but were positive in the cellobiose and Koser's sodium-citrate tests. The culture from the six-month sample was characteristic of *E. coli* since it was negative to the citrate, uric acid, and cellobiose tests but gave positive reactions on the modified Eijkman medium and on the Vaughn-Levine boric-acid medium.

Since there is a definite similarity between coliform organisms and the "soft-rot" group of *Erwinia*, as pointed out by Elrod (1942), the cultures isolated from the fresh juice were tested by the method described by Elrod for their ability to macerate carrot tissue. There was no softening of the carrot plugs after incubating for three weeks, and it was concluded that the organisms were not *Erwinia* of the type which can macerate carrot tissue. When the organisms were planted on broth containing a plug of orange which consisted of a bit of flesh of the orange and a portion of albido peel, the reaction of the broth rose from about pH 5 to 7 or above, and a very heavy growth was produced. There was no softening of the albido, although the flesh became flaccid. No definite conclusions were drawn as to whether the organisms which had utilized the citric acid and sugars in the orange tissue were *Erwinia* or *Aerobacter*. Further work is to be done along these lines and reported later.

DISCUSSION

Although numerous coliform bacteria may be isolated from the juice of "soft-rot" oranges, very few appear to belong to the "fecal" section of the coliform group. Judging from the findings on juice extracted from sound fruit under laboratory conditions, it would appear impossible to produce orange juice commercially that is free from gram-negative, nonspore-forming bacilli which ferment lactose with gas production. As indicated by Hunter (1939), there are limitations to the significance of coliforms in certain classes of raw material such as vegetables and milk; orange juice, considering its plant source and method of production, may also be added to the list. Broadly speaking, while the presence of *E. coli* in orange juice should throw suspicion on its sanitary history, no such viewpoint seems warranted from the demonstration of members of the *Aerogenes* group. It would seem therefore that if qualitative methods are resorted to in the bacteriological examination of frozen orange juice, identification of species rather than group placement is called for. In this respect it may be noted that some plant pathogens, i.e., *Erwinia carotovora* and *Erwinia phytophthora*, are gram-negative, nonsporing, facultative rods, fermenting lactose with gas production and even simulating *E. coli* on Endo and, to a lesser degree, eosin-methylene-blue agar. The conclusion of Elrod (1942), who reports that had the *Erwinia* cultures he studied been isolated in the course of a coliform investigation, they almost certainly would have been classified as aberrant coliforms, is very significant in this regard.

While counts running into millions of bacteria per cubic centimeter, such as were yielded by juice from poor fruit (No. 31), are of course to be considered excessive, the technic needed to produce juice containing only a very few thousand per cubic centimeter would probably be impractical commercially. The question of bacteriological standards for the frozen product must await the accumulation of further data before an accurate answer can be given. In this connection attention should be called to the findings of McFarlane (1942), who found that *E. coli* in frozen, unsweetened orange juice is markedly reduced in numbers after one week at 0°F. storage, although in sweetened juice the lethal effect is much less. Wallace and Tanner (1935) report that *E. coli* dies in acid medium less rapidly at a very low storage temperature than at temperatures nearer the freezing point, but the lethal effect is definite, even at temperatures below 0°F. This lethal effect has been reported by Schrader and Johnson (1934) to be comparatively rapid. These workers concluded that coliforms do not survive in frozen orange juice for a period longer than two weeks. This conclusion is at variance with the findings reported above, since completed coliform tests were obtained from juice from poor fruit (No. 31) even after eight months' storage at 0°F. One cannot conclusively explain these differences with data at hand. However, the original numbers present in the juice undoubtedly play an important part here, since a heavily contaminated juice, such as the juice from poor fruit (No. 31), would be more likely to have cold-tolerant bacteria than would a juice only lightly seeded.

CONCLUSIONS

Juice prepared experimentally from "soft rot" Valencia oranges was found to contain a microbial population approximately 2,500 times as great as juice similarly prepared from sound fruit. The coliform content was also much greater. Even after prolonged storage at 0°F., the viable organisms in the juice from unsound fruit were more numerous than those found in the freshly prepared juice from sound fruit.

A number of coliforms were still viable after eight months' storage at 0°F. in the "soft rot" juice, while no coliforms were isolated from the "sound" juice, which had a low initial coliform count, after as little as one month of freezing storage.

As a rule the coliforms isolated from the juice were characteristic of the *Aerobacter* genus. Of 46 isolates from "soft rot" only three, or 6.5 per cent, were *Escherichia,* and but one of these three appeared to be *E. coli.* Three isolates from "good" juice appeared to be *Aerobacter.*

Elimination of unsound fruit is obviously of prime importance in the production of frozen orange juice of low microbial count and coliform index.

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EDITORIAL REVIEW

FLAVOR OF MEAT

E. C. CROCKER
Arthur D. Little, Inc., Cambridge, Massachusetts

In the course of an investigation of the seasoning of meat and meat-containing dishes, it was found that the literature did not offer any adequate description of the flavor of meat alone. Accordingly, it was decided to initiate an experimental study using sensory methods and enough chemistry to interpret the findings. The studies here reported, on the flavor of beef and several other meats, were based on the critical personal observations of several workers. Initially, our objective was to find the location and nature of the flavor of beef, raw and boiled. The work expanded, study by study, to include lamb, pork, and chicken. Eventually, we arrived at the point where certain generalizations were possible. The work covered specifically the influence of boiling and low-temperature heating. It did not include pyrogenic flavor development, such as occurs at the surface of meats during broiling, frying, or roasting or in juices browning in the pan.

The work now reported comprises orientative studies of a preliminary nature, which serve to indicate the character of meat flavor. Much more detailed work in this field is obviously desirable.

FLAVOR OF UNAGED BEEF

Location and Nature of Flavor in Lean Meat: A piece of fresh, unaged, raw beefsteak was trimmed of its fat and various procedures were tested to find the one best adapted for separation of the fiber from the juice. Operations to obtain fibers without juice included pounding the meat, squeezing it in a hydraulic press, and leaching with water. A combination of these was found best, generally ending with three or more leachings of an hour each.

Raw-meat fiber treated as above, in order to separate it as completely as possible from the juice, had grayish white color, no detectable odor, and, on chewing, no taste. There was a slight sensation in the mouth of astringency. Chewing the fibrous material was productive of no more flavor than the chewing of cellulose fibers, and salivation was not stimulated.

On boiling extracted-meat fiber for about five minutes, it coagulated to a rubbery mass and developed some "meaty" flavor, not easily noticeable by smelling but released slowly on chewing. No amount of chewing made this flavor disappear, indicating its presence throughout the mass of the fiber. On boiling for 35 minutes and then cooling, distinct meaty flavor was noted in the fiber. The odor was typically that of cold roast beef, sulfury yet pleasingly fragrant. On chewing, flavor was released for as long as the fiber lasted. This flavor was identified as essentially an *odor,* for no more than a trace of taste (sweetness) could be detected when the nostrils were held. On release of the nostrils, a flood of meaty flavor was noted, in mouth, throat, and nose.

Boiling the juice originally squeezed from the meat produced very little odor and did not greatly increase the taste.

This study led to several findings:

1. In raw meat the small amount of blood-like flavor present resides in the juice only and not in the fiber.
2. No enzyme appeared to exist in the saliva capable of hydrolyzing raw-meat fiber to release any taste-producing substances during chewing.
3. Cooking developed meaty flavor, apparently owing to chemical changes taking place in the fiber rather than in the juices.
4. This meaty flavor, typical of cold roast beef or pot roast, was apparently due to volatile substance detected by the sense of smell, even though chewing was needed to release it. It was fragrant, moderately acidic, only slightly burnt, and distinctly caprylic. It was definitely sulfury.

Detailed Study of Beef Flavor: A piece of raw beef chuck of good quality, with pH of 6, was trimmed of fat and examined for odor and taste after various treatments. While cold, it had only a weak odor of blood or serum type, strongest where there was obvious blood. The taste was also weak, sweetish, salty, and generally blood-like.

When a piece of this meat was warmed in a 100°C.(212°F.) oven for 20 minutes, considerable odor appeared. It was an alkaline odor, in the chemical sense, and smelled of a mixture of amines including piperidine. It was also somewhat eggy, indicating the presence of sulfur compounds. The taste was mild and pleasant, although persistent, and was chiefly sweet and salty.

One piece of the beef was boiled for 25 minutes in water containing two per cent of citric acid (based on weight of the meat) and another piece in water containing one per cent of sodium bicarbonate. The acid-treated meat, with pH of 3.5, developed some acetic-like sourness of odor and a caprylic note. The taste was sourish and similar to that of corned beef. The alkali-treated meat, with pH of 8.87, had an aminic and sulfury odor, suggestive of both clams and eggs, with some piperidine evident. There was very weak taste.

A study was made of the changes occurring in the flavor of the meat with length of boiling and of the contribution made by bones, marrow, and fat. Tests showed that most, if not all, of the cooked-meat flavor came from the meat fiber, and that up to a period of three to three and one-half hours, the flavor increased, but after that time gradual loss occurred. This flavor was mostly odor, with a Crocker-Henderson odor number of about 4425 [1]—salty, seaweed-like, and grainy, like freshly baked bread. There was also a distinct vegetable-like character, suggesting in particular corn and celery. The taste was sweet, salty, fatty, and somewhat livery. The broth from long-cooked meat was sweet, salty, thin, slightly astringent, and gelatinous.

[1] Crocker, E. C., 1945. Flavor. McGraw-Hill Publishing Co., New York, N. Y.

Beef bones alone appeared to contribute almost no flavor but added gelatin and tallow, which gave body to preparations in which they were used.

Marrow, which is rich in low melting fat, and also the harder outside or tissue fats made no marked contribution to meat taste. Both fats had mild odors which diminished in intensity on long boiling.

A distillation equipment was set up and distillate was collected from boiling defatted beef. The pH of the meat at the start was 5.9, and that of the distillate about 9.25. The pH of the broth at the end of the five-hour distillation period was 6.05. The high pH of the distillate indicated that it contained free amines. Identifiable by odor were ammonia, a fishy-smelling amine, possibly methylamine, an indole-like "metallic" odor, and a crackery-smelling derivative that suggested piperidine. Also conspicuous in the odor, and easily proved chemically, was hydrogen sulfide. No acids could be distinguished by odor in the distillate until it was acidified by sulfuric acid. The acids, including a low aliphatic type such as acetic or propionic acid, could be noticed. This direct test indicated that the odor of cooked meat is due to a variety of chemical substances, presumably produced by fragmentation, such as deamination or decarboxylation, of amino acids simultaneously with some breakdown of the sulfur-bearing amino acid cysteine to yield H_2S and propionic acid.

A variant of the distillation procedure was the boiling of beef in two beakers, one with plain water and the other with water in which zinc oxide was suspended. This latter reagent was found able to capture most of the hydrogen sulfide without itself materially affecting the pH of the water. The beaker containing the zinc oxide smelled much "cleaner" than the one without it, though both odors were definitely meaty. The odor number with plain water was taken as 4425 and that with the zinc oxide 4323, a point less acid and two points less caprylic. Even with the hydrogen sulfide largely removed, it was not possible to judge the composition of the vapor completely, by odor, although the simultaneous presence of amines and acids was quite plain. Some of the acids presumed to be present, such as phenylacetic from the breakdown of phenylglycine or phenylpropionic from phenylalanine, might well have been present, as indicated by the odor.

The findings of this study are that cooked-beef flavor is quite complicated chemically, and consists more of odor than of taste. Indicated as present were hydrogen sulfide, amines of several kinds including a low simple form, one of the piperidine type, and possibly indole. The vegetable-type odors imply the presence of traces of volatile acids. The only elements of true taste noted were sweetness and saltiness, no doubt owing to actual blood salts. Astringency was noted in beef extracts as well as in cooked fiber.

FLAVOR OF PORK, LAMB, AND CHICKEN

Studies similar to that outlined for beef were made on pork, lamb, and chicken. The findings only are given herewith for the sake of brevity. Pork, at a pH of 6.30, proved to have a fundamental meaty character similar to that of beef, on which was superposed more sweetness of taste; much more volatile fatty acid content, ranging from acetic to the higher

fatty acids; additional bases, including an earthy-potatoey type; and more sulfury ingredients suggestive of chicken. The above observations, made on eastern pork, were repeated on choice Iowa pork, with similar findings. From the flavor of the meat it appeared that either the food or the environment of the animal had imparted certain qualities to the meat with some types giving a sour-vegetable character unless long cooked. There was also more or less of a "piggy" note present that may be characteristic of the distinctive chemistry of the animal, perhaps glandular in part, and separate from the influence of the surroundings and food.

Lamb ribs, with pH of 7, was a far more alkaline meat than beef or pork and had a marked hexylamine character even when strictly fresh. Also, there was present a strongly caprylic or pelargonic acid type of odor, characteristic of sheep and goats. (There is apparently no chemical inconsistency in acidic and basic odors both being noted simultaneously at the neutral pH of 7, since hydrolysis should be considerable with weak acids and bases.) Long cooking drove off the unpleasant acidic odor and most of the amine, and the meat then had a more delicate flavor, approaching that of beef. At no time was any sulfury odor conspicuous with lamb.

Chicken flavor was found to be even more complex than that of the red meats, varying considerably between parts of the same bird and from stage to stage in the life cycle. The breast meat tasted sourish and somewhat astringent but was mild in all birds tested. The leg meat of fowl, however, tended to be quite eggy in the upper joint and less so below, more prominently sulfury and stronger with the older birds. When a bird was held uneviscerated for some hours at room temperature, an unpleasant "gutty" odor of mercaptan or organic sulfide type was found to permeate the carcass, first appearing in the cavity, then on the inside of the top of the leg and gradually all over. The dominant features found in chicken flavor were the eggy odor and taste, especially noticeable in the skin and in the dark meat, and prominent sweetness and saltiness of taste.

NATURE OF MEATY FLAVORS

The flavor of raw meat resides mostly in the juice. It is apparently due to the sweetness and saltiness of actual blood and only incidentally and unimportantly to the presence of the bases creatine and creatinine [2] which have but weak tastes.

Most of the flavor of meat created by boiling or other low-temperature heating is presumably due to the "cracking" of amino acid units of the protein, particularly those of the fiber. We have no information from this study that indicates that the proteins are first broken down, by hydrolysis or other means, to peptones or to free amino acids before they are cracked. Possibly, the cracking takes place in the main protein chains or in the "side-chains." Meat cooked at low temperature retains all the salts and sugars noted in the raw meat, and these may be noted in the taste.

The wide variety of amines, acids, and sulfur compounds and possibly also phenols, released each in small proportion, implies that amino acid

[2] Bendall, J. R., 1946. Effect of cooking on the creatine-creatinine . . . of beef . . . J. Soc. Chem. Ind. 65, No. 85, 226.

units of several types are changed during cooking. Some of the fragments released from the amino acids have taste, generally of sweet, salty, or bitter type, but the most conspicuous flavor elements present are odors. Cooked-meat flavor is thus predominately odor.

All of the meats examined seemed to have a weak, fundamental, blood-like flavor when raw, on which was superimposed flavors distinctive of the species, food, and environment of the animal. The flavors that developed on cooking likewise were similar for the various meats, again modified by the species, food, and environment of the animal, although even these characteristics tended to be lost with prolonged cooking. The differences seemed to be more in quantity than in quality, for all red meats were found to have some of the flavor characteristics of fish and birds, and the meat of birds and fish had red-meat characteristics.

THIAMIN STABILITY IN SPRAY-DRIED WHOLE EGG[1,2]

ALLEN L. OLSEN, JOSEPH A. WEYBREW, AND
RALPH M. CONRAD
Kansas State College, Manhattan, Kansas

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During the recent war large amounts of spray-dried whole egg were produced, representing a highly concentrated form of a widely used protective food. Because of its important place in army rations and in the feeding of civilians in allied countries, the stability of the vitamins which it contains has been of considerable interest.

Klose, Jones, and Fevold (1943) have shown that riboflavin, pantothenic acid, and niacin are very stable in dried egg. On the other hand, they find that thiamin and vitamin A are destroyed rather rapidly during storage of dried egg. Similar studies (unpublished[3]) in this laboratory lead to the same conclusions. However, it was also observed that the deterioration of thiamin was greatly retarded at low moisture contents and by the addition of acid, as might be expected, since egg powder normally has a pH of 8 to 8.5. Because of recent interest in egg powder of low moisture content, Quartermaster Corps (1943), the effect of moisture content on thiamin stability has been further investigated.

EXPERIMENTAL PROCEDURE

The egg powder studied was dried in a commercial plant producing powder of approximately two per cent moisture content. The powder was received in the laboratory on the day after production and packed in nitrogen and carbon dioxide in three-pound tin cans. Higher moisture levels were produced by placing about half a pound of powder in a fruit jar with a calculated amount of water in an open test tube, the water distributing itself by vapor diffusion through the powder within a few days. The lower moisture levels were produced by vacuum redrying of the same powder. The samples of powder of different moisture contents were stored at 37°C.(98.6°F.). As a control, one jar of powder was stored at 3°C.(37.4°F.), the earlier work having indicated that no measurable change in thiamin content took place under these conditions. Moisture and thiamin content were determined in these samples after 20 weeks and 57 weeks of storage. Fluorescence, a commonly used measure of deterioration of egg powder, was also determined for reference.

The thiamin content was determined by the method of Hennessy and Cerecedo (1939) with modifications suggested by preliminary work in this laboratory and by the work of Conner and Straub (1941). Five grams of dried whole egg or 20 gm. of liquid egg was digested for 30 minutes

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[2]Supported through a grant from the National Egg Products Association.
[3]Supported as a Nutrition Fellowship by Swift and Company.

on a steam bath with 70 ml. of N/10 sulfuric acid containing 10 gm. potassium chloride. It had been found that enzymatic digestion and purification by adsorption are unnecessary. A five-ml. aliquot was taken for the thiochrome development, as directed by Hennessy and Cerecedo, except that 15 ml. of isobutanol was used and a glass-stoppered reaction vessel designed by Maisel-Gerson (supplied by Wilkens-Anderson Co.) replaced the cylinder and separatory funnel.

Moisture and fluorescent measurements were made according to the technique adopted by the Quartermaster Corps (1944).

DISCUSSION

At high moisture levels a temperature of 37°C. brings about marked changes in thiamin content (Table 1). The relationship between thiamin

TABLE 1

Thiamin Retention in Dried Egg[1] at 37°C.(98.6°F.)

Moisture	Type of pack	Thiamin		Fluorescence	
		20 wk.	57 wk.	20 wk.	57 wk.
pct.		*pct.*	*pct.*	*units*	*units*
1.29	Air pack	82	61	45	47
1.66	Air pack	76	59	49	47
3.53	Air pack	46	30	68	63
3.59	Air pack	41	27	64	64
4.37	Air pack	22	11	71	63
5.65	Air pack	12	1	76	57
2.45	Gas-packed tin	65	50	54	55

[1] Control sample stored in refrigerator contained 2.55 per cent moisture, 3.70 γ of thiamin per gm., and 18 units of fluorescence.

retention and moisture is linear within the experimental error of the determinations independent of gas packing. This would appear to contradict the conclusion of Cruickshank, Kodicek, and Wang (1945) that high temperature rather than high moisture is the cause of thiamin destruction. Our earlier data and that presented here indicate that both factors are important. It is further to be observed that a greater part of the deterioration occurs in the initial period of the storage. However, significant changes take place in the second phase of the storage as well. The values for fluorescence appear to reach a maximum in the initial period of storage. It is to be noted that whole dried eggs are alkaline, having a pH of 8 to 8.5 and that freshly dried powder is higher by approximately one unit of pH than powder stored for six months, according to Stuart Goresline, and Dicks (1942).

According to Williams and Spies (1938), thiamin in solution is quite stable at low pH but at high pH the molecule decomposes. At pH 5 to 6, for example, in a sulfite solution, thiamin undergoes a cleavage and loses its activity. In neutral and alkaline solutions it is extremely sensitive to heat.

The importance of the normally high pH of egg powder in the destruction of thiamin was demonstrated by acidifying liquid egg to a pH of 4.2 before drying. The resulting powder contained 3.24 γ thiamin and 3.3

per cent moisture. After 15 weeks' storage at 37°C. it retained 93 per cent of its original thiamin. A control sample dried from the unacidified liquid retained only 36 per cent of its original thiamin content after the same storage period.

It is interesting to compare thiamin stability in dried eggs with that in dehydrated pork. According to Rice and Robinson (1944), dehydrated pork has a very high initial thiamin content, 15 γ per gram. However, after 130 days of storage at 37°C. only 0.62 γ is retained. By interpolation it can be determined (Table 1) that eggs dried to the two-per cent moisture level required by recent Quartermaster Specifications should retain approximately 2.62 γ per gram after similar storage treatment. It should be pointed out that the dehydrated pork studied by Rice and Robinson contained a comparatively large amount of moisture (probably about six per cent). The data presented for eggs suggest that if it were practical to dehydrate pork to low moisture levels, the stability of its thiamin might be very greatly increased.

SUMMARY

The stability of thiamin in dried eggs stored at 37°C. varies inversely with moisture content from approximately one to six per cent. At moisture levels below two per cent the amount of the vitamin retained after several months' storage is greater than in dehydrated pork, indicating that when troops must be supplied largely with dehydrated foods, dried eggs may constitute an important source of thiamin.

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COMPARISON OF ANALYZED WITH CALCULATED DIETS

VINCENT TOSCANI

Russell Sage Institute of Pathology, in affiliation with the New York Hospital and Department of Medicine, Cornell University Medical College, New York, New York

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During the course of a metabolic study of immobilization on four normal men, reported by Deitrick, Whedon, and Shorr (1948), it was deemed desirable to investigate the constancy of the constituents of diets used. The study spanned a period of about two years.

Throughout the course of the experiment 12 diets were analyzed for their nitrogen, calcium, phosphorus, sodium, and potassium content. Three of the diets had a value of 2,500 calories, while nine of the diets had a value of 2,800 calories. The diets were collected over a period between August, 1944 and May, 1946.

EXPERIMENTAL PROCEDURE

The solid portions of the weighed diets were ground in a meat chopper, mixed thoroughly, and placed in a large evaporating dish. The liquid portions of the menus were then added to the ground food. The dish was then placed on a steam bath. The drying process usually required from five to seven days. The food mixture was broken up from day to day with a spatula to expose the moist cores of the lumps of food to the heat. The material was weighed, ground in a meat chopper, and quartered to insure homogeneous sampling. The quartered portion was then ground to a finer mesh for analysis.

Nitrogen was determined by a slight modification of the method of Folin and Wright (1919). A one-half-gram sample of the dried material was digested using 20 c.c. of a mixture consisting of 170 c.c. of concentrated sulphuric acid, 130 c.c. of 85 per cent phosphoric acid, and 100 c.c. of a three per cent solution of copper sulphate. Upon complete digestion the mixture in the flask was made up to 150 c.c. with water, and a few lumps of pumice were added. To the flask 100 c.c. of 40 per cent sodium hydroxide was added and 100 c.c. of the contents of the flask were distilled into standard acid.

Ten grams of the dried food was placed in a "Vycor" glass crucible and 10 c.c. of 4N sulphuric acid was added. The crucible was placed on a hot plate at moderate heat in a hood to carbonize the organic portion of the food. When carbonization was complete, the crucible was placed in a muffle furnace maintained at a temperature between 500 to 600°C.(932 to 1,112°F.). As soon as a white ash was obtained, the crucible was removed from the oven. The ash was taken up with 10 c.c. of concentrated hydrochloric acid, transferred to a 500-c.c. volumetric flask and made up to volume. This ash solution was used for the determination of calcium, phosphorus, sodium, and potassium.

TABLE 1

Weights of Constituents of Analyzed Diets

Diet No.	1	2, 7	8	3	4, 9	10	5	6, 11	12
Calories	2,490	2,794	2,799	2,499	2,802	2,812	2,486	2,796	2,805
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Apple, edible portion	.....	.....	.....	.....	.....	.....	150	150	150
Asparagus, canned	.....	.....	.....	75	75	75	.....	.....	.....
Bacon, edible portion	.....	.....	.....	35	35	35	.....	.....	.....
Beans, lima, canned	50	50	50	.....	.....	.....	.....	.....	.....
Beans, string, fresh	100	100	100	.....	.....	.....	.....	.....	.....
Bread, rye	90	120	120	.....	.....	.....	.....	.....	.....
Bread, semi-white	.....	.....	60	.....	.....	120	.....	.....	110
Bread, whole wheat	.....	.....	.....	30	60	60	60	60	60
Bread, white	60	60	.....	120	120	.....	80	110	.....
Butter, salted	80	81	81	43	45	45	72	75	75
Carrots, fresh	100	100	100	100	100	100	.....	.....	.....
Cheese, American	.....	.....	.....	.....	.....	.....	10	10	10
Cherries, canned	.....	.....	.....	100	100	100	.....	.....	.....
Chicken, edible portion	75	75	75	.....	.....	.....	.....	.....	.....
Coffee, 5% extract (c.c.)	300	300	300	300	300	300	300	300	300
Corn, canned	.....	.....	.....	50	75	75	.....	.....	.....
Cornflakes	.....	.....	.....	.....	.....	.....	25	25	25
Cream, 20% (c.c.)	30	45	45	30	40	40	30	40	40
Eggs	150	150	150	100	100	100	100	100	100
Grape juice, canned (c.c.)	.....	150	150	.....	150	150	.....	.....	.....
Grapefruit, fresh	100	100	100	75	100	100	.....	.....	.....
Grapefruit juice, canned (c.c.)	.....	.....	.....	.....	.....	.....	.....	150	150
Grapenuts	.....	.....	.....	25	25	25	.....	.....	.....
Jam, fruit	.....	.....	.....	55	52	52	.....	10	10
Lettuce, edible portion	20	30	30	20	30	30	10	30	30
Macaroni	.....	.....	.....	.....	.....	.....	30	30	30
Marmalade	30	30	30	.....	.....	.....	.....	.....	.....
Milk, fresh (c.c.)	400	400	400	400	400	400	400	400	400
Orange juice, fresh (c.c.)	.....	.....	.....	.....	100	100	100	.....	.....
Peaches, canned	100	150	150	.....	.....	.....	.....	.....	.....
Pears, canned	100	150	150	.....	.....	.....	.....	.....	.....
Peas, canned	.....	.....	.....	.....	.....	.....	90	100	100
Pineapple, canned	.....	.....	.....	100	150	150	100	150	150
Pork chop, edible portion	.....	.....	.....	90	90	90	.....	.....	.....
Potato, fresh	100	150	150	150	150	150	125	150	150
Steak, lean, edible portion	.....	.....	.....	.....	.....	.....	125	125	125
Sugar	40	32	32	35	30	30	58	59	59
Tomatoes, fresh	.....	.....	.....	.....	.....	.....	100	150	150
Tomato juice, canned (c.c.)	.....	.....	.....	100	.....	.....	.....	100	100
Wheat, shredded	30	30	30	.....	.....	.....	.....	.....	.....

Calcium was determined according to Hawk and Bergeim (1937) using 50 c.c. of the ash solution.

Phosphorus was estimated according to Fiske and Subarrow (1925) using one c.c. of the ash solution.

Sodium and potassium content of the diet was obtained by the method of Barnes, Richardson, Berry, and Hood (1945) employing the flame photometer.

DISCUSSION

The amounts in grams and cubic centimeters of the individual foodstuffs of the various diets and the total calories for each diet are presented (Table 1). The data for the calculated values for protein, calcium, and phosphorus were taken from Sherman's tables (1941). The sodium and potassium values for five per cent coffee extract, lean beef, and semi-white bread were obtained in our laboratory. The main portion of the sodium and potassium values was obtained from the tables of Mead Johnson (1946).

It is evident from the data (Tables 2 and 3) that there are considerable differences between the analyzed and calculated figures of the constituents of the individual diets, whereas the averages show good agreement with the published tables, with the exception of the potassium value (Table 4).

In view of the variations obtained in individual diets it is of the greatest importance to know exactly the nature of a food product used. Foods rich in sodium, such as cheese and salted butter, should be examined carefully as to their type. An example is Cheddar cheese and process cheese. The former has a sodium content of 540 mg. per 100 grams while the latter has a sodium value of 1,500 mg. per 100 grams.

The average sodium values for salted butter, slightly salted butter, and unsalted butter are as follows: 980, 670, and 5 mg., respectively.

It is apparent that a large error in the calculated value of the sodium content of the diet is possible if the category to which the butter belongs is not known. In order to by-pass the varying concentration of sodium in butter it is best to use the unsalted type. If the unsalted butter is not obtainable, it is a good practice to prepare butterfat that is free of salt. The butterfat is prepared by melting 0.5 kg. of salted butter in a double boiler. One liter of boiling water is added to the molten butter. The mixture is stirred to insure complete solution of the salt. The mixture is allowed to cool and is placed in a refrigerator. When the butterfat has solidified, it is skimmed off the aqueous layer. The fat is placed in a cloth and kneaded to eliminate the residual water. The kneaded mass is dipped several times in distilled water to remove the remaining traces of salt.

Another foodstuff which influences the calculated sodium content greatly is bacon. In the fresh condition it contains 760 mg. per 100 grams. On the other hand, if bacon is weighed after frying, the sodium content is 3,200 mg. per 100 grams.

An appreciable error can be introduced if a beverage such as coffee, which has no caloric value, is omitted from the calculated figures. The potassium value for five per cent coffee extract is about 90 mg. per 100 c.c. of the extract. As the average coffee consumption is about 300 c.c.

TABLE 2

Results of Analyses Compared With Figures Obtained From Published Tables[1]

Diet No.	Date of diet	Calories	Calculated							Analysis				
			Protein	Fat	CHO	Ca	P	Na	K	N_2	Ca	P	Na	K
1	8/17/1944	2,490	84.6	109.9	290.0	0.865	1.437	2.222	3.566	13.42	0.840	1.470	2.52	2.99
3	8/22/1944	2,499	85.5	110.3	291.2	0.833	1.503	2.556	3.864	12.35	0.810	1.320	2.45	3.43
5	8/30/1944	2,486	84.3	108.9	292.0	0.856	1.555	2.290	3.339	12.85	0.808	1.340	2.06	3.13
Total	.....	.....	254.4	329.1	873.2	2.554	4.495	7.068	10.769	38.62	2.458	4.130	7.03	9.55
		N_2	40.7											
						Total found				38.62	2.458	4.130	7.030	9.55
						Total calculated				40.70	2.554	4.495	7.068	10.769
						Per cent difference				—5.1	—3.8	—8.1	—0.54	—11.3

[1] Calcium, phosphorus, and nitrogen from Sherman's tables; sodium and potassium from Mead's tables.

TABLE 3

Results of Analyses Compared With Figures Obtained From Published Tables[1]

Diet No.	Date of diet	Calories	Calculated							Analysis				
			Protein	Fat	CHO	Ca	P	Na	K	N_2	Ca	P	Na	K
2	10/24/1944	2,794	90.2	114.2	351.7	0.926	1.555	2.408	4.130	14.20	0.887	1.490	2.28	3.64
4	10/17/1944	2,802	90.4	114.6	352.3	0.915	1.684	2.401	4.260	14.28	0.852	1.540	2.42	3.74
6	10/ 9/1944	2,796	90.1	114.2	351.8	0.920	1.668	2.845	4.201	14.38	0.865	1.570	3.13	3.66
7	2/27/1945	2,794	90.1	114.2	351.6	0.926	1.555	2.408	4.130	14.00	0.920	1.510	2.06	3.78
8	5/20/1946	2,799	91.4	114.2	351.6	0.930	1.577	2.408	4.130	13.95	0.950	1.520	2.63	3.51
9	3/28/1945	2,802	90.4	114.6	352.3	0.915	1.684	2.401	4.260	14.25	0.965	1.500	2.52	4.12
10	5/16/1946	2,812	92.8	114.6	352.3	0.929	1.733	2.401	4.260	13.80	0.954	1.570	2.58	3.61
11	3/13/1945	2,796	90.1	114.2	351.8	0.920	1.668	2.845	4.201	13.85	0.915	1.500	3.00	3.93
12	5/ 7/1946	2,805	92.4	114.2	351.8	0.933	1.712	2.845	4.201	13.85	0.910	1.640	2.31	3.81
Total	.....	.....	817.9	1,029.0	3,167.2	8.314	14.836	22.962	37.773	126.56	8.218	13.840	22.93	33.80
		N_2	130.9											
						Total found				126.56	8.218	13.840	22.93	33.80
						Total calculated				130.9	8.314	14.836	22.96	37.77
						Per cent difference				—3.3	—1.1	—6.7	—0.13	—10.5

[1] Calcium, phosphorus, and nitrogen from Sherman's tables; sodium and potassium from Mead's tables.

per day, it can be seen that omitting this beverage as a food makes for a loss of 270 mg. of potassium in the calculated diet.

Bassett, Elden, and McCann (1931) found that calcium in analyzed diets averaged 15.5 per cent lower and the sodium 18.6 per cent higher than the calculated data from Sherman's tables. The nitrogen ran 6.3 per cent lower, while the potassium and phosphorus showed good agreement. They suggest that the magnitude of these differences may be attributed to the geographical location from which these foods were obtained.

Our analyses showed good agreement with Sherman's tables for the average nitrogen, calcium, and phosphorus contents of the diets. The sodium value gave close agreement while the average potassium value was 10.7 per cent lower than Mead's tables.

TABLE 4

Deviation of Analyses From Calculated Values

Diet No.	Nitrogen	Calcium	Phosphorus	Sodium	Potassium
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
1	—0.8	—2.9	+2.3	+13.14	—16.2
3	—9.6	—2.8	—12.2	—4.2	—11.2
5	—4.8	—5.6	—13.8	—10.0	—6.2
2	—1.6	—4.2	—4.2	—5.3	—11.8
4	—1.2	—6.9	—8.5	+0.8	—12.2
6	0.0	—6.0	—5.8	+10.0	—13.0
7	—3.0	—0.6	—2.9	—14.4	—8.4
8	—4.6	+2.1	—3.6	+9.2	—15.0
9	—1.5	+5.5	—10.9	+4.9	—3.3
10	—7.1	+2.7	—9.4	+7.4	—15.2
11	—4.0	—0.5	—10.1	+5.4	—6.4
12	—6.3	—2.5	—4.2	—18.8	—9.3
Total	—44.5	—21.7	—83.3	—1.6	—128.2
Av. (pct.)	—3.7	—1.81	—6.9	—0.11	—10.7

Per cent difference..... | —3.3 | —1.1 | —0.7 | —0.13 | —10.7

[1] Calcium, phosphorus, and nitrogen from Sherman's tables; sodium and potassium from Mead's tables.

SUMMARY

Three diets having a value of 2,500 calories and nine diets having a value of 2,800 calories were analyzed for their nitrogen, calcium, phosphorus, sodium, and potassium contents. The individual diet analyses showed large variations in the content of the various constituents as compared with standard tables. The average differences gave good agreement with the tables with the exception of the potassium value which averaged 10.7 per cent lower than the Mead tables.

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PREVENTION OF DARKENING OF BANANAS DURING DEHYDRATION

JACOB FEIGENBAUM
Department of Food Control, Government of Palestine, Jerusalem

AND

SHALOM ISRAELASHVILI
Department of Biological Chemistry, Hebrew University, Jerusalem

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Kawereau and Fearon (1944) investigated a number of substances preventing the oxidation of vitamin C through copper. Thiourea has been found the most effective among the substances examined. Denny (1935) found thiourea prevented the darkening of fruits in addition to preserving its vitamin C content.

In this paper a report is made on the effect of thiourea and allylthiourea on the darkening of banana during dehydration.

EXPERIMENTAL PROCEDURE

Ripe bananas were cut into slices of about 2.5 mm. thickness and soaked in solutions of various thiourea concentration: 0.1, 0.2, 0.5, one, two, and three per cent for one, two, three, four, five, and six minutes. A portion of the soaked bananas was rinsed with water and, together with the unrinsed and thiourea untreated bananas, was kept at 30 to 32°C.(86 to 89.6°F.) during 24 hours in a drying oven connected with an oil-vacuum pump.

The results were obtained by comparing the thiourea-soaked bananas with the untreated bananas. The following observations were made:

(a) It seems that the darkening is lessened in unrinsed bananas.

(b) The optimal concentration for the prevention of darkening of bananas lies between one and two per cent thiourea.

(c) The soaking period, to prevent darkening during dehydration, is five to six minutes.

Tests were also carried out with allylthiourea. Slices of banana of two to four mm. in thickness were soaked for a few minutes in solutions containing 0.001, 0.005, 0.01, 0.05, 0.1, and 0.5 per cent allylthiourea, respectively, with and without rinsing. The soaked and allylthiourea untreated bananas were then dried in a vacuum oven, at 30 to 32°C. during the period of 24 hours.

The minimum solution of allylthiourea used to prevent darkening was found to range between 0.05 and 0.1 per cent.

According to these tests allylthiourea seems to be 10 times or more efficient than thiourea in preventing darkening of bananas during dehydration.

No appreciable difference has been observed in the behavior of thiourea or allylthiourea when the tests were carried out with halved bananas instead of sliced fruit.

It is claimed that thiourea has been shown to possess no specific pharmacological activity. Should this also prove true for allylthiourea, these preliminary experiments may indicate the significance of thiourea derivatives in prevention of darkening of fruits in dehydration or in the preservation of vitamin C.

These experiments were carried out in the Department of Biological and Colloidal Chemistry, Hebrew University, Jerusalem, with the generous co-operation of Prof. A. Fodor, director of the department. We acknowledge with thanks the interest taken by the Scientific Advisory Committee, War Supply Board, Government of Palestine, which has also sponsored this investigation.

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EFFECT OF STORAGE AND MICROBIAL ACTION ON VITAMIN CONTENT OF PORK. II.

E. E. RICE, E. M. SQUIRES, AND J. F. FRIED
Research Laboratories, Swift & Company, Chicago, Illinois

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It has been shown by Rice, Fried, and Hess (1946) that there are but insignificant losses of thiamin, riboflavin, pantothenic acid, and niacin from ground pork-flank muscles during storage at 3 to 5°C.(37.4 to 41°F.) for periods up to two weeks. Slight losses of thiamin and marked increases in riboflavin and pantothenic acid were observed when these samples were allowed to putrefy; however, the niacin content remained constant. With the exception of niacin, which decreased by amounts ranging from 0 to 50 per cent, there were likewise no losses of vitamins from whole pork loins during similar storage. Thus, the stability of niacin observed for whole loins differed from that obtained when ground trimmings were used.

It was postulated that these differences in niacin retention might be caused either by differences in the types of tissues involved (loin and flank muscles) or by the physical state of the sample (intact or ground). Changes in vitamin content might be explained on the basis of chemical reaction, enzyme action, or as a result of bacterial growth. This further work has been carried out in an effort to determine the factor or factors responsible for the changes mentioned above.

EXPERIMENTAL PROCEDURE

Retention of Niacin During Storage of Pork Loins: Since previous studies have shown niacin to be unstable during the storage of pork loins at 4°C.(39.2°F.) for periods of three days or more, and because many enzymatic reactions proceed in animal tissues more rapidly immediately after death than at later periods, it seemed desirable to determine the immediate post-mortem change in niacin content. To accomplish this, a 55-gram cube was dissected from the middle fore part of the loin of a pork carcass immediately after dispatch of the animal. The sample was cut into strips and dropped into ice-cold water, frozen quickly, and stored at —29°C.(—20.2°F.). The entire sampling procedure was completed in less than 10 minutes and the sample was thoroughly chilled in another five minutes so that opportunity for change was slight. It is assumed that changes did not occur in chilled and frozen samples or that they occurred very slowly.

The incision through which this sample had been removed was closed and the carcass was allowed to go through the regular pork-dressing line before the remainder of the loin was taken. A thin slice was trimmed from the cut surface where the first sample had been removed and then a similar sample was cut out, weighed, and frozen. This second sample was obtained one and a half hours after the first.

The remaining portion of the loin was sliced into alternate three-quarter-inch and two-inch pieces. All of the three-quarter-inch slices were frozen and held at —29°C. until assayed. The larger slices were wrapped separately in waxed paper and stored for one-, three-, or 10-day intervals at 4°C. before freezing. One of these slices was ground thoroughly, then wrapped and stored for 10 days. The storage conditions and niacin contents of the samples (Table 1) show that the losses take place gradually over a period of several days except in the ground sample, which retained all of its niacin.

TABLE 1

Rate of Loss of Niacin in Pork-Loin Slices During Storage

No.	Sample treatment	Storage period [1]	Niacin [2] content	Retention [3]
		hr.	*μg./gm.*	*pct.*
1	55 gm. frozen immediately	¼	114.2	.....
2	75 gm. adjacent to No. 1, room temperature	1¾	115.5	101
3	¾-inch slice, frozen immediately	1¾	114.0	.....
4	2-inch slice, between Nos. 3 and 5, stored at 4°C.	24	102.9	90
5	¾-inch slice	1¾	117.2	.....
6	2-inch slice, between Nos. 5 and 7, stored at 4°C.	72	82.6	72
7	¾-inch slice	1¾	110.9	.....
8	2-inch slice, between Nos. 7 and 9, stored at 4°C.	10(days)	79.5	74
9	¾-inch slice	1¾	104.2	.....
10	2-inch slice, adjacent to No. 9, ground and stored at 4°C.	10(days)	105.8	101

[1] All time following dispatch until sample is frozen is considered as storage. [2] Microbiological method, essentially that of Snell and Wright (1941). [3] Based on average of values found for the adjacent samples.

These findings completely confirm the previous observations and, furthermore, indicate that there are differences in niacin retentions of raw and ground meats.

Niacin Retention in Intact and in Ground Pork Muscles: In order to determine more definitely whether type of muscle or physical state of the meat was responsible for the differences in niacin retention observed between whole loins (50 to 70 per cent) and ground pork-flank muscle (100 per cent), experiments were conducted with loins from the right side of each of two pigs and the flank muscles from one. These samples were obtained a few minutes after dispatch of the animals.

The center sections of each loin were sliced into six two-inch segments. The first, third, and fifth segments of each loin were immediately ground

TABLE 2

Vitamin Retentions in Intact and Ground Pork Muscles Stored at Various Temperatures for Eleven Days [1]

No.	Sample	Condition	Thiamin	Riboflavin [2]		Niacin	Pantothenic acid
			μg./gm.	μg./gm. M.	F.	μg./gm.	μg./gm.
796 A	Loin ♂	Ground, stored at —29°C.	15.7	2.25	1.82	70.9	2.8
B	Loin	Ground, stored at 4°C.	15.3	2.19	2.00	70.4	3.2
C	Loin	Ground, stored at 20°C.	5.5 7.2	3.86	5.52	68.2	16.2
D	Loin	Whole, stored at 20°C.	15.1	2.24	1.81	55.2	3.3
797 A	Loin ♀	Ground, stored at —29°C.	17.6	1.83	1.52	107.0	5.4
B	Loin	Ground, stored at 4°C.	17.5	1.86	1.50	101.5	6.0
C	Loin	Ground, stored at 20°C.	9.2 6.4	3.82	3.62	95.0	12.6
D	Loin	Whole, stored at 4°C.	17.3	1.67	1.54	70.3	5.7
798 A	Flank muscle ♂	Ground, stored at —29°C.	10.7	2.36	2.41	69.0	6.7
B	Flank muscle	Ground, stored at 4°C.	10.7	2.33	2.25	68.5	6.9
C	Flank muscle	Ground, stored at 20°C.	4.7 7.2	3.53	4.45	64.8	16.3
805 A	Flank muscle ♀	Ground, stored at —29°C.	.....	.....	.....	47.1	.....
B	Flank muscle	Ground, stored at 4°C.	.....	.....	.....	46.1	.....
D	Flank muscle	Whole, stored at 4°C.	.....	.....	.....	37.5	.....

[1] The values indicate averages of assays of two separate samples, except in those cases where duplicates did not agree within 10 per cent. Both values are then listed. Inasmuch as failure of duplicate samples to check occurred only in the thiamin assays for putrefied samples it is probable that the losses are variable, probably depending on bacterial growth. [2] M. = microbiological; F. = fluorometric.

three times through a meat hasher, mixed thoroughly, divided into three jars, and distributed to storage at —29°C. (control), 4°C., and room temperature [hereafter designated as 20°C.(68°F.)]. The second, fourth, and sixth segments were pushed tightly together so that the cut surface of the second segment covered one end of the cut surface of the fourth segment, and the other end of the fourth segment contacted the sixth. These portions of the loins were wrapped in waxed paper and stored at 4°C. The flank muscles, being thin and irregular, were ground and divided into jars and placed at the three storage temperatures. After storage for 11 days the samples were assayed for vitamin content by the microbiological method of Snell and Wright (1941) (Table 2). In a subsequent experiment several of the thicker flank muscles were sliced, and alternate slices were stored whole and ground.

In general, riboflavin assays conducted by the fluorometric method of Peterson, Brady, and Shaw (1943) agreed with values obtained by the Snell and Strong (1939) microbiological method except on samples which had putrefied. In those cases high fluorometric values resulted. Purification of these extracts by adsorption on and elution from Florisil yielded values agreeing with the microbiological method, indicating that fluorescing non-riboflavin materials had been produced during the putrefaction.

In every case the ground samples retained 90 to 100 per cent of the niacin, while the paired samples which had not been ground retained only 65 to 80 per cent. The differences in niacin retention previously reported thus appear to have occurred because some of the tissues were ground while others were not. That loss of niacin from fresh intact muscle is not limited to pork alone is shown (Table 3) by data for muscles from animals of different species. These losses are much less pronounced than those reported for laboratory animals by Platt and Glock (1942).

TABLE 3

Niacin Retention in Intact Muscles From Different Species

Type of muscle	Conditions of storage	Niacin content		Retention
		Fresh	Stored	
		μg./gm.	*μg./gm.*	*pct.*
Pork loin	11 days at 4°C.	107.0	70.3	66
Pork flank	11 days at 4°C.	47.1	37.5	80
Chicken leg	14 days at 4°C.	62.5	49.5	79
Chicken breast	14 days at 4°C.	123.2	109.5	89
Beef tenderloin	7 days at 4°C.	98.0	90.0	92
Veal loin	7 days at 4°C.	90.0	84.0	93

Effect of Bacteria on Vitamin Content of Pork: The increases in riboflavin and pantothenic acid and possibly the losses of thiamin, which have been reported by Rice, Fried, and Hess (1946), may be due to bacterial action since the greatest changes occur at the 20°C.(68°F.) temperature. However, meat-enzyme activity or chemical reaction rates should also be greater at 20°C. than at the 4°C. temperature and hence may be involved in the changes. In an attempt to settle this question the earlier work has been continued. Ten-gram quantities of well-mixed, ground pork trim-

mings were placed in a number of 16 x 160 mm. tubes. The tubes were cotton-plugged and part of them were autoclaved for 15 minutes at 15 pounds steam pressure, thus destroying both the bacteria and the enzymes of the meat. After cooling, several of the autoclaved samples were inoculated with one drop of a mixed culture of bacteria. This mixed culture was obtained by washing the bacteria from four plain agar plates used in obtaining bacteria counts for previous samples of putrefied meat—Rice, Fried, and Hess (1946). Duplicate sets of raw, autoclaved, and autoclaved-inoculated samples were then stored at —29 and 20°C. for 10 days. Assays for vitamin contents gave values as shown (Table 4). As expected, the raw samples show loss of thiamin, increases in riboflavin and pantothenic acid, but no change in niacin content. There were no changes during storage of the autoclaved samples; thus it is apparent that direct chemical reactions are not responsible for the changes observed in the raw samples. The increases in riboflavin and pantothenic acid during the storage of an autoclaved sample which had been inoculated with a mixed culture of bacteria make it evident that bacteria can account for these changes but are not proof that bacterial action is the sole cause.

TABLE 4

Effect of Bacteria on Vitamin Content of Raw and Autoclaved Pork

Sample	Storage temperature	Vitamin content[1] after 10 days' storage				
		Thiamin	Riboflavin[2]		Niacin	Pantothenic acid
	°C.	μg./gm.	μg./gm. M.	F.	μg./gm.	μg./gm.
Raw	—29	7.45	3.07	2.81	46.5	6.9
Raw	20	2.42	4.75 3.34	6.62 4.98	44.8	18.2
Autoclaved	—29	2.96	2.90	2.87	32.5	5.1
Autoclaved	20	2.91	2.99	2.89	33.2	5.0
Autoclaved and inoculated	20	3.19	3.42	4.30	35.9	16.2

[1] The values shown are the average of analyses of two different samples for each condition except where the values differed by more than 10 per cent, in which case the separate values are indicated. [2] M. = microbiological; F. = fluorometric.

The most decisive answer to the role of the bacteria in causing changes in the vitamin content of meat during storage can be obtained by comparing sterile meat with paired samples inoculated with bacteria. Since muscle tissues are not always sterile and are invaded by bacteria almost immediately after death, Jensen (1945), some difficulty in securing sterile samples was expected. However, after several attempts, a few sterile samples were obtained.

Four- to six-inch sections of the loin were taken from chilled pork carcasses. Each section was trimmed to two and one-half to three inches in thickness and then seared on both end surfaces with a red hot spatula. Using sterile No. 9 cork borers several longitudinal sections, including both seared surfaces, were cut from the *longissimus dorsi* muscle and placed in

separate sterile tubes. One tube from each section was immediately frozen and stored at —29°C. Two others were stored at 20°C. and another was inoculated with a mixed culture of bacteria from spoiled meat and stored at 20°C. After two or three days the "sterile" samples were inspected and those which had begun to spoil were discarded. The remaining "sterile" samples were held for 14 days and then sampled for bacterial counts and for thiamin and riboflavin assays (Table 5). For all practical considerations Samples A-2 and B-2 remained sterile throughout the two-week storage period. In these the texture and color of the meat changed only slightly. There was no off-odor other than that of rancid fat.

The 96 and 88 per cent retentions of thiamin in these samples is evidence that this vitamin is stable during the storage of pork loin except when there is bacterial contamination. When spoilage occurs, there is thiamin loss. The extent of the loss appears to depend somewhat on the species of the bacteria, such as Samples B-3 and D-2, in which species of *Flavobacterium* developed, and showed slight decreases in thiamin as compared with Samples B-4 and D-3, in which *Pseudomonas fluorescens* predominated, or with Sample C-2, which contained a heavy growth of white micrococci.

The only definite increase in riboflavin occurred in those tubes which had been contaminated with a mixed culture from spoiled meat, confirming the previous indication that bacterial action is responsible for increases in riboflavin.

DISCUSSION

The observation that niacin is lost rather readily from intact muscle tissue confirms and extends previous reports of Rice, Fried, and Hess (1946), although the rate of loss is much less than that found by Platt and Glock (1942) for rat tissues. The resistance of niacin to oxidation by atmospheric oxygen and to other chemical changes makes it seem that the increases in stability which occur upon grinding must involve enzyme activities. This would seem especially true since grinding increases the exposure to oxygen and, hence, the opportunity for oxidative changes. It is recognized that in metabolism studies tissue homogenates give different enzyme responses from those of tissue slices. Usually, however, demonstrations of the difference between intact tissues and homogenates have been made with tissue slices 0.1 to 0.3 mm. in thickness as compared with homogenates in which most of the cell structure has been destroyed. In the ground-meat samples used in the niacin studies a large proportion of the cell structure remained intact as there was no cell destruction other than that caused by the use of a meat grinder with a plate having two-mm. openings. Such samples could be expected to respond more like tissue slices than like homogenates, and hence more like intact tissues. It is possible that the crushing action of the grinder ruptured enough of the cells to permit enzyme response characteristic of tissue homogenates rather than of intact tissue.

In view of the frequently demonstrated ability of bacteria to synthesize or destroy vitamins, it is not surprising to find the changes in vitamin concentrations observed during the spoilage of meat to be brought about

TABLE 5

Effect of Bacteria on Thiamin and Riboflavin Content of Pork

Sample No.	Conditions of 14-day storage	Thiamin content	Riboflavin content	Description of sample	Bacteria count per gram	Bacteria genera
		μg./gm.	*μg./gm.*			
A-1	—29°C.	11.1	1.6[1]	.....	.....	.....
A-2	Sterile tube, 25°C.	10.6	1.5[1]	Rancid but not putrid	Less than 1,000	.....
A-3	Sterile tube, 25°C.	6.0	1.7[1]	Became noticeably "off-odor" after 8 days	.....	.....
A-4	Inoculated, 25°C.[2]	0.5	>2.5[1]	Slimy; putrid odor after first six days	.....	.....
B-1	—29°C.	8.3	1.8	.....	.....	.....
B-2	Sterile tube, 25°C.	7.3	2.5	Very slight odor; pink color on bottom of tube	Less than 10,000	.....
B-3	Sterile tube, 25°C.	5.5	2.4	Meat light-colored; slight odor	3,700,000	*Flavobacterium*
B-4	Inoculated, 25°C.	0.9	3.3	Putrid in odor; glossy-white color	360,000,000	70% *Pseudomonas fluorescens* 30% *Achromobacter*
C-1	—29°C.	8.6	2.3	.....	.....	.....
C-2	Sterile tube, 25°C.	7.6	2.6	Musty odor; pink color	4,200,000,000	White micrococci
C-3	Inoculated, 25°C.	4.1	4.2	Putrid, slimy, and pink in color	63,000,000	*Bacillus*
D-1	—29°C.	8.9	2.4	.....	.....	.....
D-2	Sterile tube, 25°C.	8.3	2.5	Rancid odor; pink at bottom of tube, grey on top	4,000,000	*Flavobacterium*
D-3	Inoculated, 25°C.[2]	0.5	4.7	Putrid, slimy, and pink in color	2,000,000,000	*Pseudomonas fluorescens*

[1] Microbiological analyses. The remaining riboflavin values were obtained by a fluorometric procedure. [2] See text.

by bacterial action. It is interesting to note, however, that the enzymatic changes occurring during the storage of pork muscles do not involve changes in the concentration of thiamin or riboflavin, but that the concentration of niacin is reduced. In bacterial decomposition of the muscle the reverse is true, niacin remaining constant but the amounts of thiamin and riboflavin decreasing and increasing, respectively.

SUMMARY

The changes which occur in the concentrations of thiamin, riboflavin, niacin, and pantothenic acid in intact, ground, cooked, and sterile intact pork muscle have been determined under several storage conditions.

In intact pork muscle no changes were found in the thiamin, riboflavin, or pantothenic acid contents during storage for seven to 14 days at 3 to 5°C. The level of niacin, however, decreased gradually during the first three days' post-mortem, and then remained constant thereafter.

In ground pork muscle no changes were observed in the content of any of these vitamins until putrefaction occurred. Thus, niacin is more stable in ground tissue than in intact tissue. Cooked pork, like the ground, showed no change in vitamin content during storage that did not involve bacterial action.

During putrefaction there were increases in the riboflavin and pantothenic acid contents of muscles, decreases in thiamin contents, but no change in niacin contents. That these changes are brought about by bacterial action rather than by meat enzyme is indicated by the stability of three of these vitamins in bacteriologically sterile raw meat, and by the occurrence of the changes characteristic of putrefying meat samples when sterile meat, either raw or cooked, is inoculated with bacteria from putrefied meat.

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EFFECT OF VARIETY AND STORAGE ON CAROTENE AND TOTAL CAROTENOID PIGMENTS IN SWEET POTATOES

BOYCE D. EZELL AND MARGUERITE S. WILCOX

Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland

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Several workers have studied the effect of storage on the carotene (provitamin A) content of sweet potatoes. The results obtained varied widely, some reporting an increase, some a decrease, and others no significant change. Owing to the importance of sweet potatoes as a food crop, this subject warrants further study: first, to determine if there is an increase in provitamin A during storage; and, if so, to determine the conditions favorable for the greatest increase. This report is an attempt to answer this first question.

REVIEW OF LITERATURE

MacLeod, Armstrong, Heap, and Tolbert (1935), using biological methods, studied the effect of storage on the vitamin A values of sweet potatoes and reported that the Porto Rico and Yellow Jersey contained, respectively, three and four times as much after storage for two months or longer as that found at harvest. Later MacLeod and Utley (1939) reported substantial, but much smaller, increases in the Porto Rico variety, amounting to a 37-per cent increase during the first four months in storage and more than doubling in eight months. They reported no increase during storage in the Nancy Hall variety, and this finding was later confirmed by MacLeod (1940). Miller and Covington (1942), using chemical methods and the Porto Rico variety, reported an increase of about 47 per cent during the first month of storage and about 60 per cent in two months "after which time there is a trend for the carotene content to remain constant." Mitchell and Lease (1941), using the same variety, reported a gradual decrease during storage at room temperature and a more rapid loss at 37°C.(98.6°F.). More recently, and since this work was begun, Speirs, Cochran, Peterson, Sherwood, and Weaver (1945), also working with the Porto Rico variety, have reported that "in general, neither curing nor storage had any consistent or significant effect on carotene content."

Several factors may be responsible for the varying results. With most chemical methods some pigments that have no biological value are included with the carotene fraction, the amount varying with different methods. Ezell and Wilcox (1946) have shown that different roots of the same variety may vary over 400 per cent in carotene content, and that the stem end may contain two or three times as much as the root end of the same specimen. Loss of weight owing to loss of both moisture and dry matter may cause an apparent increase in carotene not detectable by moisture determinations.

To determine whether an apparent increase is real or only apparent requires information on the loss in weight during the period. In this study

the loss in weight during curing in storage was determined and taken into consideration in determining the increases or decreases in the provitamin A values.

MATERIALS AND METHODS

The sweet potatoes[1] used in these studies were grown at the Plant Industry Station at Beltsville, Maryland, in 1945. They were harvested, washed immediately, dried, weighed individually (the weight in grams of each potato being recorded thereon with an indelible pencil), and reweighed at time of sampling. This proved a satisfactory method for determining loss of weight and prevented rots in individual roots from upsetting the results. The analytical results were calculated back to the weight at harvest, and all results are reported on this basis in order to determine whether changes in carotene are real or only apparent. Samples for the "without curing" analyses were held at 15.6°C.(60°F.) and at 85 per cent humidity until the analyses could be made. The remainder of the roots was placed in the curing room on the day harvested and cured at 29.4°C.(85°F.) for eight or nine days, and then held at a constant temperature of 60°F. with a relative humidity of 85 per cent. All the varieties were harvested between October 8 and 11. The uncured samples were analyzed between October 15 and 23. To insure that the results obtained were not due to individual root variations, each variety and treatment was composed of 10 replicates of five roots each. Each sample analyzed consisted of 20 grams, taken in duplicate, of a composite of half of each of five unpeeled roots, split lengthwise, ground in a food chopper, and mixed thoroughly before sampling. The results of the duplicates were averaged and the amount reported is the average of the 10 replicates. The Wall and Kelley method (1943) was used in extracting the pigments and determining the carotene. The total ether-soluble pigments were determined from the petroleum-ether extract before chromatographing, and the carotene after chromatographing. Both were read in a photoelectric colorimeter using a filter transmitting between 410 and 475 millimicrons (maxima 440) and the concentrations calculated from the same concentration curve prepared from 90 per cent beta- and 10 per cent alpha-carotene. The moisture content was determined by drying the ground tissue at 70°C.(158°F.) for 24 hours in air, and then for a second 24 hours at the same temperature under vacuum. Samples for moisture were taken in duplicate, but from only two of the 10 replicates used in the carotene analysis. Five varieties were studied to determine if there were differences in varietal behavior. Samples were taken before curing, from storage shortly after curing, and at intervals thereafter, the last samples being taken in April.

RESULTS AND DISCUSSION

There are considerable differences in the behavior of different varieties (Table 1). While there was no statistically significant change in the carotene or the total pigments during curing in the parent strain of the

[1] The writers wish to express their appreciation to Dr. C. E. Steinbauer, in charge of sweet-potato investigations, for supplying the sweet potatoes used in this work.

TABLE 1

Effect of Curing and Storage on Carotene and Total Carotenoid Pigments of Sweet Potatoes Calculated to Original Weight of Roots at Time of Digging

Treatment	Date sampled	Carotene	Total pigments	Ratio of carotene to total pigments ×100	Loss in weight	Moisture
		mg./100 gm.	*mg./100 gm.*		*pct.*	*pct.*
PORTO RICO						
Without curing	Oct. 22	4.01	5.30	75.7	3.57	67.11
Shortly after curing	Nov. 20	4.07	5.16	78.8	8.61	65.90
After storage	Jan. 7	4.36	5.36	81.2	10.52	66.65
After storage	Mar. 12	4.39	5.46	80.3	11.62	68.05
After storage	Apr. 24	3.94	4.93	80.0	13.91	68.80
Difference for significance						
5-per cent level	.....	0.35	0.38	2.49	0.77	.....
1-per cent level	.....	0.46	0.51	3.32	1.04	.....
PORTO RICO UNIT 1						
Without curing	Oct. 23	3.74	4.83	77.5	3.06	67.03
Shortly after curing	Nov. 26	4.14	5.34	77.5	8.03	67.49
After storage	Mar. 14	4.27	5.21	81.8	12.68	67.03
Difference for significance						
5-per cent level	.....	0.31	0.31	4.43	0.91	.....
1-per cent level	.....	0.42	0.41	5.98	1.23	.....
NANCY HALL						
Without curing	Oct. 16	1.46	3.11	47.0	1.28	64.31
Shortly after curing	Nov. 15	1.99	2.71	73.5	6.22	63.17
After storage	Jan. 2	2.09	2.73	76.4	7.49	63.29
After storage	Mar. 6	2.02	2.74	73.7	8.62	61.92
After storage	Apr. 8	2.03	2.74	74.3	10.18	64.07
At 70°F. one month	Apr. 18	1.86	2.51	74.1	9.55	64.33
Difference for significance						
5-per cent level	.....	0.14	0.14	2.56	0.49	.....
1-per cent level	.....	0.18	0.19	3.40	0.65	.....
YELLOW JERSEY						
Without curing	Oct. 18	0.089	0.685	13.0	3.31	68.25
Shortly after curing	Nov. 16	0.248	0.929	26.7	7.39	68.38
After storage	Jan. 4	0.400	1.216	32.8	10.75	66.23
After storage	Mar. 7	0.472	1.265	35.9	14.06	70.56
After storage	Apr. 9	0.617	1.510	40.8	18.31	71.46
At 70°F. one week	Mar. 25	0.590	1.562	37.7	17.26	66.70
At 70°F. two weeks	Apr. 1	0.524	1.382	37.9	17.23	72.30
Difference for significance						
5-per cent level	.....	0.060	0.097	2.36	0.79	.....
1-per cent level	.....	0.080	0.129	3.14	1.06	.....
SOUTHERN QUEEN						
Without curing	Oct. 15	0.052	0.377	13.7	4.65	62.39
Shortly after curing	Nov. 28	0.119	0.409	29.2	7.18	60.95
After storage	Jan. 8	0.144	0.498	29.0	9.75	64.24
After storage	Mar. 13	0.127	0.445	28.5	11.06	62.55
After storage	Apr. 23	0.134	0.391	34.3	11.17	64.21
At 70°F. one month	Apr. 19	0.151	0.462	32.8	14.34	64.04
Difference for significance						
5-per cent level	.....	0.018	0.036	3.92	0.82	.....
1-per cent level	.....	0.024	0.048	5.22	1.09	.....
TRIUMPH						
Without curing	Oct. 19	0.037	0.417	8.7	3.42	62.29
Shortly after curing	Nov. 19	0.129	0.449	28.8	7.77	61.41
After storage	Jan. 5	0.141	0.513	27.5	9.63	62.35
After storage	Mar. 8	0.132	0.475	27.8	10.16	60.85
After storage	Apr. 15	0.178	0.543	32.7	13.75	60.50
At 70°F. one month	Apr. 18	0.187	0.592	31.6	12.06	61.01
Difference for significance						
5-per cent level	.....	0.010	0.027	1.42	0.63	.....
1-per cent level	.....	0.014	0.036	1.89	0.84	.....

Porto Rico variety, there was an increase in the ratio of carotene to total pigments. There was a slow increase in carotene during storage, but by March 12 the increase was significant only at the five-per cent level. The total pigments changed but little during this time. Six weeks later both carotene and total pigments were significantly lower although the potatoes were still sound and normal in external appearance. The potatoes lost approximately 14 per cent in weight, although the moisture-solids ratio varied comparatively little.

A smaller lot of Unit 1 Porto Rico was studied. At harvest this group of Unit 1 averaged a little less in carotene and total pigments than did the parent strain. It increased significantly, however, in both carotene and total pigments while curing, and thereafter contained about the same amount as the parent strain. The ratio of carotene to total pigments increased slightly but not significantly during storage. It might be added, however, that the ratio of carotene to total pigments was very low in one replicate of the sample taken after curing. No explanation for this low result could be found, but had this replicate been omitted from the calculations, the difference required for significance would have been smaller and the increase then would have been significant at the five-per cent level.

With Nancy Hall there was a definite and highly significant increase in carotene and decrease in total pigments during curing. The ratio of carotene to total pigments increased over 50 per cent. During storage there was not much change in either carotene or total pigments, but after one month at 21.1°C.(70°F.), when sprouting was quite pronounced, there was a loss in both carotene and total pigments. The sprouts were removed from the sprouted roots and were not included in the tissue analyzed. No analyses were made of the sprouts so it is not known whether or not the carotenoids lost were transferred from the roots to the shoots. Such a transfer would presuppose a change from a water-insoluble to a water-soluble product and thus, temporarily at least, a destruction of the carotenoid pigments. After the roots were removed from the 60°F. storage to 70°F., they were sprinkled with water and kept covered with wet cloths to induce sprouting. Under these conditions the roots lost less weight than did those held at 60°F. continuously. The same was true in some of the other varieties.

The Yellow Jersey at harvest was low in both carotene and total pigments. During curing there was a significant increase in both, and the increase continued throughout the storage period. The Southern Queen and the Triumph have light-colored flesh and are low in carotene and total pigments. The carotene content of these varieties increased appreciably during the curing and in storage, but the starting level was so low that they remained comparatively poor sources of provitamin A.

While there is an increase in carotene during curing and storage, and with some varieties, like the Yellow Jersey, storage increases the provitamin A value severalfold, variety is the dominant factor in determining the provitamin A values of sweet potatoes. This is shown graphically (Fig. 1) in which the carotene values of the different varieties at harvest and in storage are plotted on the same scale. The Yellow Jersey had the greatest

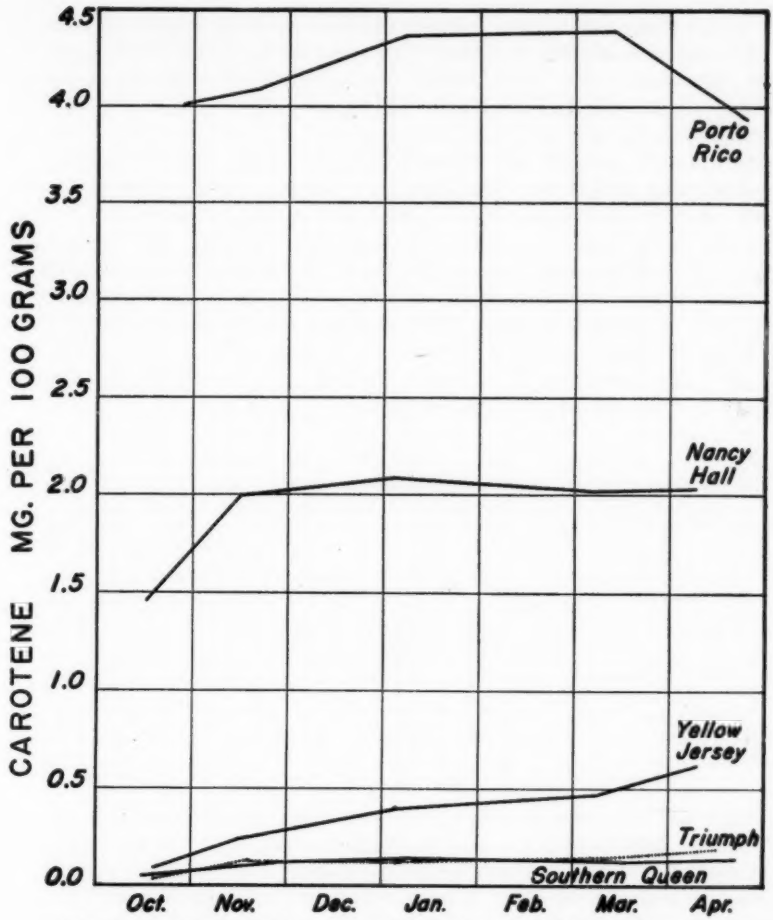

FIG. 1. Carotene content of five varieties of sweet potatoes at harvest and during storage.

percentage increase in provitamin A value, increasing almost sevenfold, yet Nancy Hall at harvest contained over twice as much carotene as the Yellow Jersey at its highest value, and the Porto Rico had about twice as much as Nancy Hall.

It was somewhat surprising to find that Unit 1 Porto Rico at harvest contained slightly less carotene and total pigments than did the Porto Rico variety, since Unit 1 was originally selected from the parent strain for its high carotene content. That this was the case suggests that continued care in selecting propagating stock is necessary to maintain strains high in carotene content. If such selection is effective, then it would seem probable that the same method might be used to develop strains with even higher carotene content.

It is of interest to note among the five varieties studied that as the flesh becomes more deeply colored the carotene increases much more rapidly than either total pigments or the non-carotene pigments (total pigments — carotene = non-carotene pigments). This is shown (Table 2); total pigments, carotene, and non-carotene pigments (average values at harvest and in storage) are recorded for the several varieties in the order of increasing flesh color. The Southern Queen, which averaged least in both carotene and non-carotene pigments, has been represented by 1.00. The values for other varieties are then shown as multiples of Southern Queen; Porto Rico contained 36 times as much carotene as Southern Queen, 12 times as much total pigments, and only 3.5 times as much non-carotene pigments. Ezell and Wilcox (1946) reported that in general this relationship between flesh color and high carotene content held true within a variety also, thus enabling one to select roots of high provitamin A values by choosing those with deeply colored flesh.

TABLE 2

Total Pigments, Carotene, and Other Pigments in Five Varieties of Sweet Potatoes and Their Relative Rate of Increase With Increasing Depth of Color of Flesh

Variety (in order of increasing flesh color)	Total pigments	Carotene	Non-carotene pigments	Multiples of Southern Queen		
				Total pigments	Carotene	Non-carotene pigments
	mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*			
1. Southern Queen	0.424	0.115	0.309	1.00	1.00	1.00
2. Triumph	0.479	0.123	0.356	1.13	1.07	1.15
3. Yellow Jersey	1.121	0.365	0.756	2.64	3.17	2.44
4. Nancy Hall	2.806	1.918	0.888	6.61	16.67	2.87
5. Porto Rico	5.242	4.154	1.088	12.36	36.12	3.52

The loss in weight in the different varieties ranged from 10.2 per cent in Nancy Hall to 18.3 per cent in Yellow Jersey. The relative humidity of the storage room was mechanically controlled at 85 per cent, which is probably higher than that in most sweet-potato storage houses. The loss in weight reported here may therefore be somewhat less than usually occurs.

In previous studies other workers have usually calculated the carotene on the basis of weight when analyzed, disregarding the loss in weight during curing and storage. The effect of this practice is shown (Table 3); the results for Porto Rico are recalculated to the weight at time of sampling and entered alongside those based on weight at harvest. Three erroneous conclusions are indicated under these conditions: (1) a significant increase in total pigments when there really was none; (2) an increase in carotene, significant at the one-per cent level instead of at the five-per cent level, (3) a significantly greater (at the five-per cent level) amount of carotene in late storage than at harvest although the actual weight of carotene was slightly less in late storage than at harvest.

In another test to determine if there is an actual increase in carotene during curing and storage a series of paired half-roots was used, one half

TABLE 3

Effect of Loss in Weight During Curing and Storage on Apparent Carotene Content of Porto Rico Sweet Potatoes

Treatment	Date sampled	Carotene based on		Total pigments based on	
		Weight at harvest	Weight when analyzed	Weight at harvest	Weight when analyzed
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Before curing	Oct. 22	4.01	4.16	5.30	5.49
Shortly after curing	Nov. 20	4.07	4.45	5.16	5.65
After storage	Jan. 7	4.36	4.86	5.36	5.99
After storage	Mar. 12	4.39	4.96	5.46	6.18
After storage	Apr. 24	3.94	4.58	4.93	5.73
Difference for significance					
5-per cent level	.....	0.35	0.39	0.38	0.43
1-per cent level	.....	0.46	0.52	0.51	0.57

of each root being tested before curing and the other half after curing or after curing and storage. While the behavior of roots with such large wounded areas may not be entirely comparable to whole roots, the results are of interest. For these studies 15 roots of Porto Rico and 10 of Nancy Hall were split lengthwise. The carotene content of one half of each root was determined immediately and the other half was cured before determination. In these tests the half-roots were weighed and treated as described for the whole roots. Special precautions were taken with these cut roots during curing to insure high humidity to hasten wound cork formation, but in spite of these precautions some of the roots failed to heal properly. For the carotene analyses the entire freshly harvested half-root was ground and five samples taken from each half unless otherwise noted (Table 4). With the cured and stored halves the dried, suberized, cut surface, down to normal tissue, was removed and discarded before grinding. The carotene content was calculated back to the weight at harvest as in the other tests. The results (Table 4) show significant increases in the carotene content in seven of the nine pairs of Porto Rico half-roots analyzed and in three of the six pairs of Nancy Hall. Total pigments increased significantly in six of the Porto Rico half-roots, but in only one of the Nancy Hall. Half-roots that did not heal properly, as indicated by discolored streaks in the flesh after storage, were discarded.

From the results reported it appears evident that, under the conditions prevailing in this work, there is a real increase in the carotene content either during curing or in storage. In most of the varieties studied, the increases were highly significant. Only in the old Porto Rico strain having a high carotene content at harvest was the increase not significant at the one-per cent level. However, since there were highly significant increases in the majority of the paired halves of this variety and in Unit 1 Porto Rico, a selection from the Porto Rico variety, it seems probable that an increase occurs in the old Porto Rico too.

It should be remembered that the values given are calculated back to the weights at harvest. The increases noted, therefore, are not caused by

concentration of the original amounts present owing to the loss of moisture and solids. They are due to the formation of more carotene molecules either from carotenoid pigments other than carotene or from a precursor of some other type. In a variety in which a large increase in total pigment value occurred it seems likely that part of this at least was formed from colorless precursors. It should be stated, however, that the data regarding apparent changes in the total pigments are probably less accurate than those for carotene for which the filter was selected. The total pigment values are certainly of less significance from a nutritional standpoint, since most of the carotenoids, other than the carotenes, have no known vitamin value.

TABLE 4

Effect of Curing and Storage on Carotene and Total Carotenoid Pigments in Paired Half-Roots of Sweet Potatoes

Carotene					Total pigments		
Before curing		After curing			Before curing	After curing	Difference
Date analyzed	Amount	Date analyzed	Amount	Difference [1]			
			PORTO RICO				
	mg./ 100 gm.		*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Oct. 25	4.009	Nov. 5	4.543	+.534 [1]	4.973	5.698	+.725 [1]
Oct. 25	3.252	Nov. 5	3.659	+.407 [1]	4.333	4.888	+.555 [1]
Oct. 25	4.252	Nov. 5	4.569	+.317 [1]	5.412	5.772	+.360 [1]
Oct. 29	3.432	Nov. 13	3.642	+.210 [1]	4.537	4.554	+.017
Oct. 30	4.595	Nov. 13	4.620	+.025	5.964	5.700	−.264 [1]
Oct. 25	3.450	Jan. 3	4.115	+.655 [1]	4.419	5.138	+.719 [1]
Oct. 29	2.852	Jan. 3	3.427	+.575 [1]	3.928	4.163	+.235 [1]
Oct. 29	4.044	Jan. 10	3.721	−.323 [1]	5.377	4.674	−.703 [1]
Oct. 29	3.890	Jan. 10	4.051	+.161 [2]	5.082	5.233	+.151 [1]
			NANCY HALL				
Nov. 1	1.962	Nov. 13	2.232	+.270 [2, 3]	2.763	2.901	+.138
Nov. 1	2.691	Nov. 13	2.749	+.058 [4]	3.529	3.299	−.230
Oct. 31	2.194	Jan. 10	2.549	+.355 [3, 5]	3.004	3.414	+.410 [1]
Oct. 31	2.126	Jan. 10	2.099	−.027	2.958	2.952	−.006
Oct. 31	2.812	Mar. 18	2.422	−.390 [1]	3.744	3.177	−.567 [1]
Oct. 31	1.661	Mar. 18	1.745	+.084 [1]	2.303	2.271	−.032

[1] Significant at the one-per cent level. [2] Significant at the five-per cent level. [3] Sample run in duplicate. [4] Sample run in triplicate. [5] Sample run in quadruplicate.

Kemmerer and Fraps (1943) have reported that carotene prepared by the usual methods, including the one used here, may contain several fractions, some of which may have no biological activity. Unfortunately, the method they used is not adapted to rapid routine analysis and this prevents its use where large numbers of samples are to be analyzed. In a later paper Kemmerer, Fraps, and Meinke (1945) reported that the "crude carotene" of three samples of raw sweet potatoes tested (variety not named) was equivalent to 88 per cent beta-carotene.

Sweet potatoes after harvest are still living organisms carrying on normal, though restricted, life processes, even to the initiation and devel-

opment of adventitious buds and new plants, thus starting a new life cycle. It is not particularly surprising, therefore, that changes should take place in the carotenoid pigments. If we knew the immediate precursors of carotene and other carotenoids and the factors governing their behavior, the increase in carotene might be as readily understood as the increase in sugars from the stored starch. The fact that there was an increase in the carotene-total pigment ratio in the different varieties during curing and storage is of interest. In some cases it was more than doubled and in no case was there a decrease in this ratio during curing or early storage. This fact suggests that other carotenoid pigments are formed first and perhaps serve as the immediate precursors for carotene. If this is true, then the widely varying results reported in the literature become more readily understandable. If an experiment was begun while most of the pigments were still in the pre-carotene stage, then an increase in carotene would be indicated as the carotene stage was reached. However, if most of the carotenoid pigments had reached the carotene stage before the experiment was started, then little or no increase might be found. Maturity may thus be the controlling factor in determining whether an increase in carotene during storage occurs or not. Unfortunately we have no satisfactory method of determining the maturity of sweet potatoes. Anderson, Cochran, Edmond, Garrison, Wright, and Boswell (1945) have reported significantly less carotene in sweet potatoes from late plantings than from earlier plantings, although Kimbrough, Fieger, and Lewis (1946) attributed little difference to time of planting provided the roots were of marketable size. Size of root or time of planting, however, may not be an indicator of maturity. Other factors may be more important. A study of the conditions affecting the carotene content of sweet potatoes is being continued.

SUMMARY

The carotene, total pigments, percentage of moisture, and weight of five varieties of sweet potatoes were determined at harvest, after curing, and at intervals during storage.

There was an absolute increase in the carotene content during curing and storage in all the varieties studied. In four of the five varieties the increase was significant at the one-per cent level.

After curing, halves of individual roots showed significantly increased amounts of carotene over the corresponding halves analyzed before curing.

The carotene behavior varied widely in different varieties. In Nancy Hall the increase was short and rapid, in Porto Rico it was slow and gradual, while in Yellow Jersey carotene continued to increase rather rapidly throughout the storage period.

As a source of provitamin A, sweet potatoes may be an excellent, medium, or poor source of supply, depending primarily upon the variety. In the five varieties tested the depth of color of the flesh proved to be a reliable indication of the provitamin A value. As the total pigments increased, carotene increased much more rapidly than the other pigments.

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BROWN DISCOLORATION IN MALTED PROCESS CHEESE[1]

I. HLYNKA AND E. G. HOOD

Division of Chemistry and Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada

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Malted cheese is a blend of process cheese with malt syrup. On prolonged storage, without refrigeration, as on the shelves of retail stores, its rich yellow-orange color is gradually changed into a dull orange-brown. The manufacturers sought an explanation of the color deterioration which was objectionable from the viewpoint of merchandising. The flavor of the cheese, however, remained acceptable. Some of the circumstances which were associated with the appearance of the discoloration were the change from an opaque tin-foil to a transparent cellophane wrapper, use of pasteurized malt, and a higher processing temperature of the cheese.

A study of the defect indicated an interaction of the amino acids and proteins of the cheese with aldose sugars in the malt. Frankel and Katchalsky (1937), in studying this type of reaction, showed that it was accompanied by an increase in titratable acidity and a consequent lowering of pH.

Accordingly, comparative packages of malted cheese were stored at 4.4 and 26.7°C.(40 and 80°F.). While at the lower temperature the cheese retained its color, at the higher storage temperature discoloration became perceptible after one month and increased in intensity thereafter. At this time titratable acidities and pH values were determined (Table 1). The titratable acidities are on a per-gram dry-weight basis. It may be readily seen that the titration values are definitely higher and the pH values significantly lower in discolored cheese as compared with normal cheese.

As a further confirmation that the interaction of amino acids with aldoses was the chemical basis of the discoloration, this defect has been successfully reproduced in the laboratory by the addition of chemically pure glucose to ordinary process cheese followed by prolonged storage at 80°F. Also a fluorometric determination on a NaCl extract of acetone-defatted, discolored, malted cheese by the method of Pearce, Thistle, and Reid (1943) gave a higher fluorescence value than for normal malted cheese. Other instances in which the introduction of aldose sugars to milk products resulted in brown discoloration upon heating have been reported by Ramsey, Tracy, and Ruehe (1933) and by Tracy and Edman (1942); and the chemistry of nonenzymatic browning has engaged the attention of chemists in several fields, American Chemical Society (1946).

On storing malted cheese which was prepared with raw and pasteurized malt, no appreciable difference in the discoloration was noted. Since malt

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for other purposes is now generally pasteurized, this product is used for the manufacture of malted cheese. A higher processing temperature was temporarily adopted because of a shortage of suitable wrapper coating during the war. Experiments revealed a slightly greater rate of color deterioration in cheese processed at the higher temperature. Since then, suitable wrapper coating has made it possible to return to the original processing temperature. Nevertheless, the defect, actual or potential, still exists although it has been overcome to some extent by more frequent servicing of retail stores. The cellophane wrapper, although more revealing, has now been adopted because of its greater eye appeal.

TABLE 1

Titration and pH Values of Normal and Discolored Malted Cheese After Four Months' Storage

No.	Normal (stored at 40°F.)		Discolored (stored at 80°F.)	
	ml. $\frac{N}{10}$ NaOH	pH	ml. $\frac{N}{10}$ NaOH	pH
1	4.7	5.88	8.7	5.72
2	4.0	6.00	7.1	5.67
3	4.4	5.98	7.4	5.65
4	4.4	5.98	7.4	5.73

Webb (1935) and Kaufman (1946) have shown sulfur dioxide to be effective in preventing the browning reaction. Whether this is due to the well-known property of bisulfite of combining with aldehyde groups and thus blocking the reaction of aldoses with amino compounds has not been established but is an interesting possibility.

Sodium bisulfite calculated to give a concentration of 500 p.p.m. SO_2 was added to a commercial batch of malted cheese. When initially assayed by the Monier-Williams method, Association of Official Agricultural Chemists (1945), it was found to contain 160 p.p.m. SO_2. Regular half-pound packages of normal and sulfited malted cheese were then stored at 40 and 80°F. for a period of 10 weeks. At 80°F. good color was retained in the treated cheese throughout the storage period. The SO_2-free comparative samples, however, showed a pronounced discoloration at the end of five weeks. Analysis of the treated cheese at the end of the trial period showed practically no decrease in the SO_2 concentration at 40°F., but a decrease to 58 p.p.m. at 80°F. The cheese held at 80°F. was, therefore, still protected against discoloration in spite of the favorable conditions for accelerated deterioration. It is reasonable to conclude, then, that under less drastic temperature conditions in the usual retail channels malted process cheese can be protected from discoloration by the addition of sulfites or sulfur dioxide.

The use of sulfur dioxide in food products is, of course, regulated by the various departments of public health. Proper cognizance should, therefore, be taken of the appropriate regulations should commercial application of the above scientific findings be contemplated.

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SURVIVAL OF *SALMONELLA* IN RECONSTITUTED EGG POWDER SUBJECTED TO HOLDING AND SCRAMBLING[1]

MATHILDE SOLOWEY AND ELEANOR J. CALESNICK

Microbiology Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture

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An increasing amount of evidence has accumulated relating to the contamination of spray-dried whole-egg powder with *Salmonella*—Gibbons and Moore (1944); Schneider (1945, 1946); Solowey, McFarlane, Spaulding, and Chemerda (1947); and Solowey, Spaulding, and Goresline (1946). This evidence points to the possible occurrence of *Salmonella* food-poisoning outbreaks. The danger would be enhanced should there be any delay in the use of reconstituted egg (RE) prepared from contaminated powders, particularly if the material were held for prolonged periods of time at temperatures which might be encountered in home, institution, or army field kitchens. Furthermore, it may be anticipated that the usual cooking procedures would not always suffice to destroy the *Salmonella* organisms which might be present.

The investigations reported here were designed for two purposes: (1) to study the survival of *Salmonella* in both artificially and naturally contaminated, reconstituted egg, and (2) to determine the effect of "scrambling" on the destruction of *Salmonella* present in contaminated egg liquid.

EXPERIMENTAL PROCEDURE

Powdered egg was reconstituted in the proportion of one part of powder to three parts of sterile distilled water, by weight. Uniform suspensions were obtained by mixing thoroughly in a mechanical mixer, or, in a few early experiments, with an electric stirrer and glass beads. After preliminary mixing of the egg and water (three minutes), aliquot amounts of the test bacterial suspension were added, when so desired, to give a stated number of contaminating cells. The egg melange and organisms then received an additional one-minute agitation in the mechanical mixer. The *Salmonella* strains used were all originally isolated from spray-dried whole-egg powder.

Viable-cell counts were obtained by plating suitable serial dilutions of the RE in duplicate or triplicate on tryptone-glucose-extract agar (APHA formula). The plates were incubated for 48 hours at 37°C.(98.6°F.).

Qualitative tests for the presence of *Salmonella* were made by one or both of the following procedures, according to the design of the experiment: (1) 10 gm. of RE were introduced into 50 ml. of Selenite-F (B-B-L) broth, incubated for 18 to 24 hours at 37°C., then streaked out on *Shigella-*

[1] This investigation was undertaken in co-operation with the Poultry Husbandry Section, Iowa Agricultural Experiment Station, Ames, and the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois.

Salmonella (SS) (Difco) and bismuth sulfite (Difco) agars. Suspected *Salmonella* colonies appearing on either of the two differential media were tested with "O" group antisera; (2) suspected *Salmonella* colonies appearing on the tryptone-glucose-extract agar plates were tested with the "O" group antisera. In doubtful cases, single colonies were picked for complete biochemical and serological study in order to confirm the tentative identification of *Salmonella*.

Quantitative tests for the number of *Salmonella* present in RE were made by a modification of Hoskins' (1940) Most Probable Numbers (MPN) procedure used to determine the number of coliform organisms in water samples. Five 10-ml., 1-ml., and 0.1-ml. amounts each of a given sample of RE were introduced into appropriate quantities of Selenite-F (10, 2, 1 ml.) and incubated at 37°C. for 18 hours. The contents of each tube were then streaked out on SS and bismuth sulfite agars. Suspected *Salmonella* colonies from these plates were presumptively identified by means of the "O" group antisera. Estimations of the number of *Salmonella* present per sample were made by reference to the appropriate table in Hoskins' table of Most Probable Numbers.

Two methods were used for scrambling RE: (1) a "beaker" method, as employed by the Dairy and Poultry Branch, War Food Administration (now Poultry Branch, Production and Marketing Administration) (1945) in preparing egg powder for palatability tests; that is, the RE was cooked in a beaker in a boiling-water bath (with stirring) to the consistency of a soft to moderately soft scrambled egg; (2) a "skillet" method, so designated because the RE was scrambled with constant stirring over a gas flame in a cast-iron skillet greased with a vegetable-oil shortening. Temperature readings were taken from the central portion of the stirred egg mass with an all-metal thermometer. In order to reduce subjective variables, all cooking tests were made by the same person.

An amount of RE approximately equivalent to one egg (60 gm. RE) was used for each test scramble except in certain experiments in which the approximate equivalent of six eggs (360 gm. RE) was used.

Examination of scrambled egg for viable *Salmonella* was made in the following manner: The entire "scramble" (60 or 360 gm.) was mixed while still hot with an equal volume of Selenite-F in the mechanical mixer just long enough to break up the particles of egg (one minute), then transferred to a sterile container for incubation at 37°C. The incubated material was then streaked out on SS and bismuth sulfite agars, and suspected colonies appearing on the incubated plates were presumptively identified in the manner already stated.

MULTIPLICATION OF *SALMONELLA* IN RECONSTITUTED EGG POWDER

Effect of Holding on Multiplication of Salmonella Artificially Introduced or Naturally Present in Reconstituted Egg: Egg powders with low initial bacterial content (<10 to 600 organisms per gram of powder) were reconstituted and inoculated with suspensions of *S. oranienburg* to give final concentrations, for individual experiments, ranging from 170 to 23,900 organisms per gram of RE. The artificially contaminated RE was dispensed

TABLE 1

Multiplication of Salmonella[1] *in Reconstituted Egg Powder*

Holding period	Holding temp.	RE[2] artificially contaminated with *S. oranienburg* — Experiment										RE naturally contaminated with *Salmonella* — Experiment	
		1	2	3	4	5	6	7	8	9	10	1	2
hr.	°*C.*												
0	....	170	900	1,260	8,300	11,100	13,000	15,000	15,500	21,900	23,900	100	13,600
2	5	130	.....	.....	.....	.....	.....	.....	15,100	.....	.....	90	.....
	25	280	3,100	1,630	2.5×10^4	1.8×10^4	1.8×10^4	2.1×10^4	3.1×10^4	7.8×10^4	3.0×10^4	130	1.1×10^4
	37	710	6,800	3,730	9.3×10^4	7.3×10^4	2.2×10^4	8.8×10^4	1.1×10^5	8.3×10^5	2.7×10^5	100	2.8×10^4
	45	260	1.5×10^4	6,560	2.1×10^5	1.7×10^5	5.8×10^4	2.4×10^4	1.2×10^5	1.3×10^6	3.4×10^5	60	7.1×10^4
	55	.....	750	.....	.....	.....	0	.....	.....	160	.....	.. ...	12,000
4	5	190	.....	.....	.....	.....	.....	.....	17,500	.....	.....	.....	.....
	25	910	8,000	5,430	1.4×10^6	1.2×10^5	3.8×10^4	1.4×10^4	1.2×10^5	5.7×10^5	9.2×10^4	300	2.5×10^4
	37	4.6×10^4	1.5×10^5	1.6×10^5	1.1×10^7	5.2×10^6	2.1×10^6	5.9×10^6	5.8×10^6	2.5×10^7	3.6×10^6	1,300	1.5×10^6
	45	1,460	7.4×10^5	7.0×10^5	3.5×10^7	1.2×10^7	2.1×10^4	1.0×10^4	7.6×10^6	6.4×10^7	6.1×10^6	600	4.3×10^6
	55	.....	830	.....	.....	.....	0	$<1.0\times10^4$	.....	120	.....	.....	1,700
6	5	170	.....	.....	.....	.....	.....	.....	16,000	.....	.....	130	.....
	25	5,700	4.0×10^4	1.6×10^4	9.6×10^6	5.4×10^5	.....	.....	9.4×10^5	2.4×10^6	5.5×10^5	3,000	.....
	37	1.8×10^6	5.7×10^6	4.5×10^6	2.1×10^8	1.8×10^8	.....	.....	2.1×10^8	3.5×10^8	1.5×10^8	6.0×10^4	.....
	45	2.0×10^4	1.5×10^7	9.9×10^6	5.6×10^8	4.0×10^8	.....	.....	3.3×10^8	3.8×10^8	3.3×10^8	1.3×10^4	.....
	55	.....	760	.....	.....	.....	.....	.....	.....	80	.....	.....	.....
24	5	450	.....	.....	.....	.....	.....	.....	10,200	.....	.....	100	.....
	25	9.9×10^8	9.7×10^8	8.8×10^8	1.3×10^9	1.6×10^9	9.9×10^8	7.9×10^8	2.1×10^9	2.3×10^9	1.9×10^9	1.2×10^7	6.1×10^8
	37	9.7×10^8	1.2×10^9	2.5×10^9	2.5×10^9	3.9×10^9	1.6×10^9	1.2×10^9	5.9×10^9	3.1×10^9	2.6×10^9	1.4×10^8	1.3×10^9
	45	5.1×10^8	1.1×10^9	1.2×10^9	1.5×10^9	1.4×10^9	1.0×10^6	5.1×10^5	1.2×10^9	9.2×10^8	1.3×10^9	8.0×10^9	3.3×10^9
	55	.....	50	.....	.....	.....	0	<100	.....	0	.....	.....	10

[1] Viable-cell count per gram RE. [2] RE = reconstituted whole-egg powder.

into sterile cotton-stoppered glass containers and held at 5, 25, 37, 45, and 55°C.(41, 77, 98.6, 113, and 131°F.). RE prepared from powders naturally contaminated with *Salmonella* was similarly dispensed and held under the same experimental conditions. Viable-cell counts were made at the beginning of the holding period and after two, four, six, and 24 hours at each temperature. Representative colonies appearing on the tryptone-glucose-extract agar serial dilution plates were confirmed as *Salmonella* by slide agglutination with the appropriate "O" group antisera.

Results of 10 experiments with artificially contaminated RE (Table 1) indicated that multiplication of *Salmonella* was progressive at 25, 37, and 45°C., although more rapid at the last two temperatures. After 24 hours at 5°C., the viable-cell count remained approximately the same as at the initial hour. *Salmonella* organisms were not recovered after two hours or longer at 55°C. but were recovered from RE held at the four lower temperatures.

Results of two experiments with naturally contaminated RE reveal a time-temperature relationship similar to that observed for RE artificially contaminated with *Salmonella*, despite the differences in the ratio of *Salmonella* to the microbial flora present in both types of RE.

Effect of Holding on Number of Salmonella Present in Naturally Contaminated, Reconstituted Egg: Three *Salmonella*-positive powders were reconstituted and held under the same experimental conditions at four of the five test temperatures (25, 37, 45, and 55°C.). Immediately after reconstitution and again after two, four, six, and 24 hours at each temperature, samples of RE were removed for quantitative (MPN) estimations of the number of *Salmonella* present. Multiplication was again progressive at 25, 37, and 45°C., although more rapid at the last two temperatures (Table 2). It was not practical to attempt a more definitive estimation of the most probable numbers of *Salmonella* present after the longer holding periods because of the large quantities of RE and the numerous dilution tubes that would have been required.

Effect of Holding on Ratio of Salmonella to Viable-Cell Count of Naturally Contaminated, Reconstituted Egg: Four batches of RE, each prepared from different *Salmonella*-positive powders, were held at room temperature [27°C.(80.6°F.)]. Immediately after reconstitution and at four, six, and 24 hours, portions were removed for viable-cell counts and for quantitative *Salmonella* determinations. As indicated (Table 3), the original *Salmonella* counts were approximately the same in the four batches of RE, although the viable-cell counts ranged from 5,300 to 25,300 organisms per gram (mixed flora). With the exception of the first sample, multiplication of *Salmonella* was rapid, and by the fourth and sixth hours, relatively high. Again, because of the limitations in the MPN technic mentioned earlier, the ratio of *Salmonella* to viable-cell count could not be satisfactorily estimated at each time period.

Effect of Holding on Incubation of Salmonella-Negative, Reconstituted Egg: It was thought that subminimal numbers of *Salmonella* not detected by routine examination of egg-powder samples might multiply sufficiently in RE to facilitate their isolation if RE prepared from these powders was

held. Accordingly, three batches of RE prepared from *Salmonella*-negative powders were held at the test temperatures. At zero, two, four, six, and 24 hours, portions were removed for enrichment in Selenite-F and the subsequent qualitative examination for the presence of *Salmonella*. None of the samples yielded *Salmonella* even after 24 hours' incubation.

EFFECT OF SCRAMBLING ON SURVIVAL OF *SALMONELLA* IN RECONSTITUTED EGG POWDER

Effect of Scrambling on Survival of Salmonella Present in 60 Grams of Artificially Contaminated, Reconstituted Egg: Batches of RE were artificially inoculated with suspensions of one of three *Salmonella* types—

TABLE 2

Multiplication of Salmonella in Naturally Contaminated, Reconstituted Egg Powder

Holding period	Holding temp.	Number of *Salmonella* per 100 grams RE [1]		
		Experiment		
		1	2	3
hr.	°C.			
0	.....	2	5.6	6.1
2	25	.....	17	7.8
	37	.....	33	23
	45	.....	27	49
	55	.....	0	.....
4	25	4.5	49	13
	37	240	79	180
	45	<4	33	540
	55	0	0	.....
6	25	.....	79	27
	37	.....	350	>1,600
	45	.....	>1,400	430
	55	.....	0	.....
24	25	>1,600	>1,600	>1,600
	37	>1,600	>1,600	>1,600
	45	>1,600	>1,600	>1,600
	55	0	0	.....

[1] RE = reconstituted whole-egg powder; estimated by modification of Hoskins' (1940) Most Probable Numbers (MPN) technique.

S. oranienburg, S. pullorum, or a heat-resistant strain of *S. senftenberg.* In individual experiments the inoculum of the contaminating organisms ranged from 800 to 8,000 cells per gram of RE. Control scrambles were made from the contaminated RE immediately after preparation. The remainder was then divided, and portions were held at room temperature (27°C.) and refrigerator temperature (5°C.). After two, four, six, and 24 hours, test scrambles were made in duplicate. Whenever sufficient egg powder was available, four to six scrambles per test interval were made. The procedures for qualitative *Salmonella* examination of the "before scrambling" RE and the scrambled egg have been described in the section

TABLE 3

Salmonella[1] *and Viable-Cell Counts Before and After Scrambling Naturally Contaminated, Reconstituted Egg Powder*

Sample No. and scrambling method	Holding time at room temperature															
	0 hour				4 hours				6 hours				24 hours			
	Before scrambling		After scrambling		Before scrambling		After scrambling		Before scrambling		After scrambling		Before scrambling		After scrambling	
	Sal. per 100 gm. RE[2]	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE	*Sal.* per 100 gm. RE	Viable-cell count per gm. RE
1 (Beaker)	350	5,300	0	0	49	7,800	0	4,530	35	16,300	0	386	250	2.4×10^{8}	360	6.4×10^{6}
2 (Skillet)	240	11,200	280	1,076	>1,600	147,000	0	730[3]	>1,600	2.3×10^{8}	42	18,500	>1,600	9.9×10^{8}	240	2.9×10^{8}
							0	693[3]			26	11,060				
3 (Skillet)	920	12,200	158	6,000	>1,600	218,000	13.6	1,440	>1,600	2.4×10^{8}	260	496	>1,600	1.1×10^{9}	46	249,000
							0	676			0	546				
4 (Beaker)	240	25,300	0	<10	240	36,600	0	3,660	>1,600	201,000	0	110	>1,600	1.0×10^{10}	340	986,000
							0	1,260			0	1,060				

[1] Estimated by modification of Hoskins' (1940) Most Probable Numbers (MPN) technique. [2] RE = reconstituted whole-egg powder. [3] Results of duplicate tests.

on experimental procedure. It should be recalled that the entire contents (60 gm.) of the scrambled egg were examined for *Salmonella*.

Salmonella strains were recovered from 84 of 287 scrambles prepared from RE held at room temperature (27°C.) and nine of 28 scrambles prepared from RE held at refrigerator temperature (5°C.). *Salmonella* organisms were recovered from scrambles made after each storage-time interval, regardless of the *Salmonella* type used to contaminate the RE. It should be emphasized that positive scrambles were obtained not only from RE held for as short a time interval as two hours but also immediately after reconstitution. Three of the zero-hour *S. pullorum*-positive scrambles and, similarly, two of the *S. senftenberg*-positive scrambles were obtained in experiments in which the original *Salmonella* inoculum was as low as 800 to 1,500 organisms per gram of RE, indicating perhaps that the density of the *Salmonella* inoculum is only one of several factors to be considered in the survival of *Salmonella* in scrambled egg.

TABLE 4

Distribution of Salmonella-Positive Scrambles Made From Artificially Contaminated, Reconstituted Egg Powder Held at Room Temperature (27°C.)

Holding period	Over-all total		RE [1] contaminated with					
			S. oranienburg		*S. pullorum*		*S. senftenberg*	
	Number of scrambles	Number positive for *Salmonella*	Number of scrambles	Number positive for *Salmonella*	Number of scrambles	Number positive for *Salmonella*	Number of scrambles	Number positive for *Salmonella*
hr.								
0	74	17	30	1	24	13	20	3
2	61	4	17	2	24	1	20	1
4	51	8	7	2	24	4	20	2
6	50	12	6	6	24	3	20	3
24	51	43	6	5	25	22	20	16
Total.....	287	84	66	16	121	43	100	25

[1] RE = reconstituted whole-egg powder.

Of the combined total of 315 scrambles prepared from RE held at room or refrigerator temperatures, 153 were made by the beaker method, and 162 by the skillet method of scrambling. The cooking times and temperatures for the scrambles prepared by the two methods ranged from less than one to seven minutes for the 60-gm. quantity. Generally speaking, the cooking times for the scrambles prepared by the skillet method were of slightly shorter duration, although it is evident that the duration of time and temperature attained by this method were due in large measure to the amount of heating given the skillet as well as the shortening prior to the addition of egg.

Forty-one of the 153 beaker scrambles and 52 of the 162 skillet scrambles were positive for *Salmonella*. The distribution of the positive scrambles obtained by each method with respect to the holding time of the RE showed a marked similarity. Positive scrambles were recovered at all ranges of the time-temperature interval. Indeed, two of the scram-

bles from which *Salmonella* were recovered were cooked as long as seven to eight minutes at temperatures of 69 to 72°C.(156.2 to 161.6°F.).

Effect of Scrambling on Survival of Salmonella Present in 360 Grams of Artificially Contaminated, Reconstituted Egg: Four batches of RE were prepared in the usual manner, and to each one suspensions of *S. oranienburg* were added to make a final concentration of 5,000 to 8,000 organisms per gram. Scrambles were made from the contaminated RE immediately after preparation and again after two, four, six, and 24 hours at 27°C. The cooking times ranged from one minute and 10 seconds to 17 minutes and 11 seconds; the temperatures ranged from 56 to 75°C.(132.8 to 167°F.). All the scrambles were made by the skillet method. In every instance the entire scramble (360 gm.) was examined for *Salmonella* in a manner similar to that already described for the examination of the 60-gm. equivalent.

Salmonella organisms were recovered from 12 of 40 scrambles. The distribution of the positive scrambles was as follows:

Holding time	Number of scrambles	Number of *Salmonella*-positive scrambles
hr.		
0	8	3
2	8	2
4	8	2
6	8	0
24	8	5
Total	40	12

Effect of Scrambling on Salmonella Present in Naturally Contaminated, Reconstituted Egg: Fifty-six *Salmonella*-positive powders were used in this phase of the investigation. All powders were tested immediately prior to use in the experiments to confirm the presence of *Salmonella.*

Four hundred and seventy-eight scrambles were prepared, 295 from RE held at 27°C. for two, four, six, and 24 hours, and 183 from RE held at 5°C. for similar time periods. Seventy-one of the 295 scrambles were positive for Salmonella. None of the scrambles prepared from the RE held at 5°C. yielded *Salmonella.* The distribution of the positive scrambles was as follows:

Holding time	Number of scrambles	Number of *Salmonella*-positive scrambles
hr.		
0	70	5
2	24	0
4	70	15
6	70	14
24	61	37
Total	295	71

Two hundred and thirty-eight of the 478 scrambles were prepared by the beaker method and 240 by the skillet method; 36 of the former and 35

of the latter were *Salmonella* positive. Among the *Salmonella* types recovered from these scrambles prepared from naturally contaminated RE were strains of *S. oranienburg, S. tennessee, S. senftenberg, S. newington,* and *S. anatum.*

Effect of Scrambling on Viable-Cell Count as Well as Number of Salmonella Present in Naturally Contaminated, Reconstituted Egg: Four batches of RE, prepared from as many naturally contaminated powders, were stored at 27°C. At zero, four, six, and 24 hours, portions were removed for viable-cell counts and quantitative *Salmonella* determinations before and after scrambling. Variability of the results obtained is shown (Table 3). The ratio of the numbers of *Salmonella* present before and after scrambling does not in any way reflect a consistent picture when considered in relation to the time of incubation of the stored RE or in relation to the viable-cell count. The Most Probable Numbers method for determining the number of *Salmonella* present in egg powder containing a mixed bacterial flora gave only a rough quantitative estimate. Negative findings do not preclude the presence of *Salmonella.* Tests made on several scrambles that were negative for *Salmonella* by quantitative examination (MPN) indicated that *Salmonella* were indeed still present, as determined by slide agglutination of picked single colonies appearing on tryptone-glucose-extract agar plates used to determine viable-cell counts.

DISCUSSION

From these investigations it may be seen that *Salmonella* strains multiply rapidly in RE held at temperatures ranging from 25 to 45°C. The presence of other organisms in the menstruum undergoing multiplication at the same time and under the same conditions of incubation was not sufficient to prevent entirely the recovery of *Salmonella.* That their presence must exert an inhibiting effect, however, is evident.

Experimental data of Haines and Elliot (1944) and Gibbons, Moore, and Fulton (1944) and those reported here show that rehydrated egg powder is a good medium for the growth of *Salmonella* at temperature ranges of 25 to 45°C. In fact, Haines and Elliot state that because of the rapid multiplication of *Salmonella* "the period of rehydration should not exceed 4 hours." Our own observations substantiate this statement. It would seem that RE held beyond this time constitutes a critical potential hazard. While holding is not advisable, it is evident that, if unavoidable, it should be at refrigeration temperatures.

Salmonella strains were recovered from test scrambles prepared from artificially and naturally contaminated, reconstituted egg powders. Recovery was without doubt dependent on a combination of several variables: the original concentration of the contaminating organisms, duration of the holding period of RE prior to scrambling, holding temperature, time and temperature of scrambling, and type of scramble. In considering the latter, the use of a soft to moderately soft scramble as a criterion for the evaluation of the presence of *Salmonella* in the heated product may in a large measure account for the positive scrambles obtained in these studies as compared with the negative results reported by Gibbons and Moore

(1944). On the whole, their scrambles were cooked at higher temperatures for longer times than those reported here. However, we were able to recover *Salmonella* from a scramble cooked 17 minutes and 11 seconds at 56°C. (skillet method). The original *Salmonella* concentration of RE in this instance was only 1,300 organisms per gram.

Salmonella species were recovered more frequently from scrambles prepared from artificially contaminated RE than from scrambles prepared from naturally contaminated RE. The heavier initial *Salmonella* concentration in artificially contaminated RE as well as the increased ratio of *Salmonella* to the remaining bacterial flora undoubtedly contributed to this greater frequency.

Salmonella strains were recovered from scrambles prepared by both beaker and skillet methods. Little difference was noted in the number of positive scrambles obtained by the two methods. It is evident that the common methods of scrambling cannot be depended upon to destroy these organisms in contaminated egg.

In general, *Salmonella*-positive scrambles occurred more frequently as the holding period of contaminated RE was lengthened. This was due, no doubt, to the progressive multiplication of *Salmonella* organisms in RE.

The recovery of *Salmonella* from scrambles prepared from RE held at 5°C. was probably primarily dependent upon the magnitude of the initial contamination, assuming that conditions of testing and examination were the same.

The demonstrated ability of *Salmonella* to multiply rapidly in RE at temperatures of 25 to 45°C., together with the fact that these organisms may frequently survive scrambling temperatures, points to the desirability of using egg powder immediately after its reconstitution.

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EFFECT OF OLD-FASHIONED AND MODERN METHODS OF COOKING ON RETENTION OF NUTRIENTS IN VEGETABLES. II. SNAP BEANS

MARY S. EHEART AND MATTIE LOU SHOLES
Department of Agricultural Chemistry,[1] Virginia Agricultural Experiment Station, Blacksburg, Virginia

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In spite of education to the contrary, snap beans are still prepared in many sections by the "old-fashioned method" in which the beans are cooked several hours in a water, salt, and fat-meat mixture. It was thought worth while to determine the effect of this method on retention of nutrients as compared with recommended procedures.

Very little study has been made of old-fashioned methods as discussed in an earlier paper by Eheart and Sholes (1948). A few studies of beans cooked by present-day methods have been made. Beans cooked in the waterless cooker were compared by Brinkman, Halliday, Hinman, and Hamner (1942) with those cooked in an open kettle. The beans cooked in the open kettle were found higher in calcium than those cooked in the waterless cooker and about the same in phosphorus and ascorbic acid. The retention of ascorbic acid was 51 per cent in the open kettle and 49 per cent in the waterless cooker. Similar results were found by Ireson and Eheart (1944) when the tightly covered and open-kettle methods were compared for cooking beans. It was found that there was no significant difference between the two methods for retention of ascorbic acid since the open-kettle method retained 57.5 per cent and the tightly covered kettle, 58.8 per cent. Noble and Waddell (1946) also found no significant difference in the ascorbic acid content of green beans cooked in the open kettle and in the tightly covered kettle since the percentage retention of ascorbic acid was 62 to 74 for different varieties cooked by the open kettle and 66 to 68 for those varieties cooked in the tightly covered kettle. Mack, Tapley, and King (1939) reported a retention of 62 and 66 per cent of the ascorbic acid content of two varieties of beans when cooked in the open kettle. The loss of ascorbic acid was due primarily to solution. Thus, the studies in which the open-kettle and waterless-cooker methods have been compared do not seem to show much difference in retention of ascorbic acid in beans.

Several studies have been made in which beans were cooked in water in ratios of from 1:1 to 4:1 but these methods do not simulate the open-kettle or waterless-cooker methods—Farrell and Fellers (1942), Phillips and Fenton (1945) and Van Duyne, Chase, Fanska, and Simpson (1946). Retentions of ascorbic acid in these studies were from 74 to 78 per cent. In one study by Van Duyne *et al.* (1946) the retention of ascorbic acid was lowered from 78 to 66 per cent when the ratio of beans to water was

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changed from 2:1 to 1:2 but the cooking time was the same. It was generally found true that vegetables cook more quickly in a large amount of water than in a small amount. Thus, it may be seen that studies in which all variables are controlled except one, such as amount of cooking water, probably do not simulate general practices as used in the home.

In the present study, green beans were studied for retention of nutrients when cooked by commonly used methods, viz.: the open-kettle method, tightly covered-kettle method, the modified old-fashioned method, and the old-fashioned method. Thus, the comparisons made were not of the effect of one variable but of the composite of variables involved in different cooking methods.

EXPERIMENTAL PROCEDURE

Statistical Design of Experiment:[2] A total of 63 cookings of beans was made—nine replications of six cooking methods and nine comparable samples of the raw beans. A sufficient amount of beans was bought from the market for one complete replication and this procedure was repeated on nine different days. In this way a large variation in the raw beans was introduced into the experiment so that the differences owing to cooking method could be studied by the analysis of variance in relation to differences owing to the daily variation of beans as purchased from the market.

Cooking Methods: Details for the six methods of cooking are given (Table 1). Two methods of preparation, Frenched and snapped, were compared in both the open-kettle method, using a large quantity of water, and the tightly covered-kettle method, using a minimum of water. In the modified old-fashioned method the fat meat was cooked two hours in the salted water prior to addition of the snapped beans which were then cooked until just tender. In the old-fashioned method the snapped beans, cold water, salt, and fat meat were brought to boil, the heat reduced, and cooked for three hours.

TABLE 1

Description of Cooking Methods Used

Method of cooking	Weight of beans cooked	Method of preparation of beans	Volume of water used	Salt used	Amount of fat meat used	Average cooking time
	gm.		*ml.*	*gm.*	*gm.*	*min.*
Tightly covered kettle	600	Frenched	100	8.5	.....	38.4
Tightly covered kettle	600	Snapped	100	8.5	.....	32.7
Open kettle	600	Frenched	1,900	22.0	.....	27.1
Open kettle	600	Snapped	1,400	17.0	.....	29.1
Modified old-fashioned	600	Snapped	1,200	14.0	140	29.8
Old fashioned	1,200	Snapped	1,600	13.0	200	180.0

Analytical Methods: Samples from all raw and cooked beans were dried in fruit jars in circulating air at 72°C.(161.6°F.), ground in a hammer mill, and analyzed for moisture, ether extract, calcium, and phosphorus

[2] Dr. Boyd Harshbarger and Dr. D. B. DeLury, statisticians in the Virginia Agricultural Experiment Station, were responsible for the plan of experiment and for the analysis of the data.

by the official methods of the Association of Official Agricultural Chemists (1940).

Ascorbic acid was determined in the raw and cooked beans and in the cooking water by the Morell method (1941) in which three per cent HPO_3 was used as the extractant and the ascorbic acid present was measured by the amount of decolorization of indophenol dye as determined by the photoelectric colorimeter.

Carotene was determined in the raw and cooked beans by a modification [3] of the Moore and Ely method (1941). By this method the sample was dropped into boiling 95 per cent ethyl alcohol, allowed to stand five minutes, and ground in a Waring blendor with a foaming mixture of 57 per cent alcohol in petroleum ether (B.P. 30-60°). After complete washing of the residue and alcohol layer with petroleum ether, the solution of pigments in the petroleum-ether layer was washed with water until free of alcohol. A mixture of dicalcium phosphate and supercel was used as the adsorbent for pigments other than carotene and the final solution of carotene was read at 440 millimicrons in the photoelectric colorimeter. The colorimeter had been calibrated with crystalline carotene.

Judging the Cooked Beans: Samples of the cooked beans were warmed and judged by three experienced judges whose average scores were used in the analysis of the data.

RESULTS AND DISCUSSION

The effect of the method used in reporting data upon results obtained from studies of the effect of cooking method on the composition of vegetables was discussed in an earlier paper by Eheart and Sholes (1948). It was shown that the absorption of NaCl and loss of dry matter during cooking caused the results expressed on the dry basis to be invalid. Likewise, changes in water content during cooking made conclusions inaccurate when data were reported on the basis of 100-gram portions. It was concluded that the most accurate method of comparing different cooking methods was on the total-lot basis.

In the previous study on cabbage by Eheart and Sholes (1948), NaCl was determined only on the samples cooked by one of the methods which showed an increase of dry matter during cooking. In the present study, all samples were analyzed for NaCl and the data were recalculated on the basis of the corrected dry matter. The dry matter changes during cooking are reported on both the corrected and uncorrected basis (Table 2). It may be seen that the percentage retention of dry matter during cooking by various methods varied from 83.1 to 112 on the uncorrected basis and from 72 to 100.7 on the corrected basis. Thus, it is evident that in experiments on cooking vegetables dry matter should always be corrected for NaCl absorption before these data are used. On the corrected basis it may be seen that the beans cooked in the open kettle, whether Frenched or snapped, retained the least amount of dry matter during cooking, 78.7 and 72 per cent, respectively. The modified old-fashioned method caused

[3] Personal communication by Dr. Mary Speirs, Georgia Agricultural Experiment Station, Experiment, Ga.

only a slight loss of dry matter, for the beans retained 94.1 per cent of the original amount. Beans cooked in the tightly covered kettle or by the old-fashioned method did not lose significant amounts of dry matter.

The 600-gram lots of raw beans varied in weight after cooking from 518.2 to 610.1 grams. The beans cooked in the tightly covered kettle, whether Frenched or snapped, decreased in weight significantly, weighing 530.1 and 518.2 grams, respectively, after cooking. Those cooked by the old-fashioned method also weighed less after cooking, 556.3 grams. Beans cooked by the open-kettle method or by the modified old-fashioned method did not change significantly in weight during cooking.

TABLE 2

Retention of Dry Matter, Cooked Weights, and Judges' Scores of Green Beans Cooked by Different Methods

Method of cooking	Number of samples	Retention of dry matter		Cooked [1] weights	Judges' scores	
		Uncorrected for NaCl	Corrected for NaCl		Color [2]	Total palatability [3]
		pct.	*pct.*	*gm.*		
Tightly covered kettle, Frenched	9	112.0	100.7	530.1	2.7	13.6
Tight covered kettle, snapped	9	106.8	97.4	518.2	2.6	12.7
Open kettle, Frenched	9	83.1	78.7	610.1	3.9	14.7
Open kettle, snapped	9	91.6	72.0	593.9	3.7	13.5
Modified old-fashioned	9	102.5	94.1	610.1	3.2	12.4
Old-fashioned	9	106.0	96.8	556.3	1.5	12.0
L.S.D.[4]	....	4.7	4.2	14.4	0.4	1.0

[1] Equivalent to 600 gm. raw. [2] Perfect score = 4. [3] Perfect score = 16. [4] Least significant difference at the five-per cent level.

These significant dry-matter losses and changes in weight of the total lots during cooking would cause errors in the data reported on the per gram, dry basis or the per 100 grams, wet basis, as has been discussed. Therefore, all data in the present study are reported only on the cooked-lot basis.

The beans, cooked by the various methods, were judged for palatability by three trained judges. Color was scored highest (Table 2) in beans cooked in the open kettle, next highest in those cooked by the modified old-fashioned method, lower in those cooked in a tightly covered kettle, and lowest in beans cooked by the old-fashioned method. The average scores ranged from 1.5 of a possible four for the color of those cooked by the old-fashioned method to 3.9 for the Frenched beans cooked in an open kettle. Similar results had been reported by Brinkman *et al.* (1942), who found that beans cooked in the waterless cooker were judged inferior to those cooked in an open kettle, especially in color and flavor.

In the present study the total palatability score was highest for the Frenched beans cooked in an open kettle, 14.7 of a possible 16, and lowest for those cooked by the old-fashioned method, 12. The Frenched beans in both cases were judged somewhat higher in palatability than the corresponding snapped samples. However, the differences in total palatability among all methods were not as great as were those for cabbage,

Eheart and Sholes (1948). The calcium, phosphorus, ascorbic acid, and carotene contents of green beans cooked by the different methods are given (Table 3).

The calcium content of the raw beans and beans cooked by all the methods studied did not differ significantly and the values varied only from 170 to 198.2 mg. per lot (equivalent to 600 grams of raw beans).

The phosphorus content of cooked green beans varied with the method of cooking. Frenched beans cooked in a tightly covered kettle were highest, containing 243.2 mg. of phosphorus per cooked lot, while those Frenched and cooked in an open kettle contained the least amount, 158.9 mg. The only significant loss from the raw beans was in those Frenched and cooked in an open kettle.

TABLE 3

Calcium, Phosphorus, Ascorbic Acid, and Carotene Contents of Green Beans Cooked by Different Methods When Reported on Total-Lot Basis [1]

Method of cooking	Number of samples	Calcium	Phosphorus	Ascorbic acid	Carotene
		mg.	*mg.*	*mg.*	*mg.*
Raw	9	181.8	222.9	69.7 [2]	2.15
Tightly covered kettle, Frenched	9	198.2	243.2	35.2	2.13
Tightly covered kettle, snapped	9	173.6	234.2	42.9	1.83
Open kettle, Frenched	9	170.3	158.9	18.7	2.22
Open kettle, snapped	9	182.0	207.7	30.8	2.06
Modified old-fashioned	9	170.0	221.8	23.1	2.50
Old-fashioned	9	178.1	230.9	13.3	2.51
L.S.D.[3]	....	N.S.	17.7	8.3	0.30

[1] Equivalent to 600 gm. raw. [2] The L.S.D. value does not apply to the raw beans in this case, which is obviously higher than the cooked samples. [3] Least significant difference at the five-per cent level.

All methods of cooking beans caused a significant loss of ascorbic acid (Table 3) from the raw beans which contained 69.7 mg. per 600-gram lot. The cooked beans ranged from 42.9 mg. for the snapped lots cooked in a tightly covered kettle to 13.3 mg. for those cooked by the old-fashioned method. The Frenched beans tended to retain less ascorbic acid than snapped beans since those cooked in the tightly covered kettle retained 35.2 mg.; those cooked in an open kettle, 18.7 mg.; while snapped beans cooked by these methods had 42.9 and 30.8 mg. per lot, respectively. For snapped beans, the methods ranked as follows: the tightly covered kettle with a retention of ascorbic acid of 42.9 mg., the open kettle and modified old-fashioned methods with retentions of 30.8 and 23.1 mg., respectively, and the old-fashioned method with a retention of only 13.3 mg. in the total lots.

The percentage distribution of ascorbic acid in the cooked beans, cooking water, and that oxidized is given (Table 4). The percentage retention of the original ascorbic acid found in the cooked beans varied from 61.6 in the snapped beans cooked in a tightly covered kettle to 16.7 for those cooked by the old-fashioned method. The greatest solution in the cooking water, 42.4 per cent of the original amount, was from Frenched beans cooked in a large amount of water in an open kettle. The cooking water

from beans cooked by the other three methods (open kettle, snapped; modified old-fashioned; and old-fashioned), in which a sizeable amount of water was used, contained about the same amount of ascorbic acid, 16.3 to 18.1 per cent of the total. Loss of ascorbic acid to oxidation was highest in the old-fashioned method, 66.9 per cent, and lowest in Frenched beans cooked in the open kettle, 29.7.

TABLE 4

Original Ascorbic Acid Remaining in Cooked Beans, in Cooking Water, and That Lost by Oxidation

Method of cooking	Number of samples	Cooked beans	Cooking water	Oxidized
		pct.	*pct.*	*pct.*
Tightly covered kettle, Frenched	9	49.5	.....	50.5
Tightly covered kettle, snapped	9	61.6	.....	38.4
Open kettle, Frenched	9	27.9	42.4	29.7
Open kettle, snapped	9	45.0	16.3	38.7
Modified old-fashioned	9	30.5	18.1	51.5
Old-fashioned	9	16.7	16.4	66.9

The carotene content of the raw beans was 2.15 mg. per 600-gram lot while beans cooked by the several methods varied from 1.83 to 2.51 mg. (Table 3). The only significant loss of carotene during cooking was in snapped beans cooked in the tightly covered kettle, which contained 1.83 mg. of carotene. Beans cooked by either the modified old-fashioned or old-fashioned methods were significantly higher than the raw beans and contained 2.50 and 2.51 mg., respectively. Previous reports had been made of such apparent increases in carotene during cooking. Zscheile, Beadle, and Kraybill (1943) reported that two varieties of raw spinach contained 48.8 and 49.2 micrograms per gram of B-carotene, whereas after cooking the value was 52.4 μg. It was thought possible that this increase in carotene in the present study was due to its more complete extraction from the cooked samples owing to added fat in the old-fashioned and modified old-fashioned methods.

A further study was made in which 24 samples of raw beans were analyzed for carotene, half of which contained an amount of added fat comparable to that present in the beans cooked by the old-fashioned and modified old-fashioned methods. The data were submitted to the analysis of variance which showed that there was no significant difference between the mean of the samples containing fat and that of those containing no fat. Thus, the reason for these significant increases in carotene during cooking are at present unknown. They may possibly be due to enzymatic destruction in the raw samples.

It has been frequently suggested that most of the nutritive value of vegetables cooked by the old-fashioned method is to be found in the "pot liquor." Keller and Minot (1941) concluded that the "pot liquors" from turnip greens and cabbage were excellent sources of ascorbic acid since they contained, respectively, 0.23 and 0.11 mg. per ml. In the present study, a comparison of the calcium, phosphorus, and carotene contents of raw beans and those cooked by the old-fashioned method (Table 3) showed

that there were no significant losses of these nutrients to the cooking water. Although there was a large loss of ascorbic acid when beans were cooked by this method, the cooking water contained 0.08 mg. per ml. which was only 16.4 per cent of the original amount. Most of this vitamin, 66.9 per cent, had been oxidized. There may have been present larger amounts of nutrients other than those determined in this study, such as thiamin, riboflavin, etc. in the "pot liquor." However, from the nutrients analyzed in the present study, it would seem that the "pot liquor" from beans may be considered only a fair source of ascorbic acid and of no value in respect to carotene, calcium, and phosphorus.

Although the analyses were not included in the calculation of data, the beans cooked by the old-fashioned method were allowed to stand overnight in a cool place and warmed over the next day since this is a common practice in many homes. The warmed-over beans were found to have lost all their ascorbic acid content but there was no significant change in the carotene values.

The discussion of results thus far has been from the standpoint of the effect of method of cooking on the nutritive value and palatability of the beans since that was the original purpose of the experiment. As has been stated, however, a large variation in the beans was introduced into the experiment by purchasing them from the local market, a plan which would simulate average household practices. The importance of this variation may be seen by comparing the mean squares for replication (daily market variation) with those values for method of cooking (Table 5).

TABLE 5

Mean Squares for Effect of Replication (Daily Market Variation) and Cooking Method on Composition and Palatability of Green Beans

Factors studied	Replication		Cooking method		Mean square ratio
	D/F	Mean square	D/F	Mean square	
Percentage retention of dry matter	8	129.3	6	1,137.2	8.8 [1]
Cooked weight of beans	8	4,851.0	5	14,835.0	3.1 N.S. [2]
Color	8	0.3	5	6.9	23.0 [1]
Total palatability score	8	0.9	5	8.4	9.3 [1]
Ca/per total lot	8	20,298.4	6	859.2	23.6 [1]
P/per total lot	8	13,326.5	6	8,075.3	1.7 N.S.
Ascorbic acid per total lot	8	1,644.0	5	1,083.9	1.5 N.S.
Carotene per total lot	8	1.3	6	0.5	2.6 N.S.

[1] Significant at the 0.5-per cent level. [2] N.S. = nonsignificant.

In the case of such factors as retention of dry matter during cooking, color, and total palatability scores, the effect of cooking method was much higher than the daily variation in the beans. In the case of color, for instance, the ratio of the mean square for method of cooking to the mean square for replication was 23, which showed that the method of cooking had a much greater effect on this factor than replication.

The effect of method of cooking on the nutritive value of green beans was in no case greater than the effect of replication or daily market varia-

tion (Table 5). For calcium, the ratio of the mean square for replication to that for method of cooking was 23.6, thus showing how much more important was the daily market variation than the method of cooking on the calcium content of cooked beans. In the case of phosphorus, ascorbic acid, and carotene the mean squares for replication, although higher, were not significantly different from those for method of cooking, and the ratios of the mean squares were 1.7, 1.5, and 2.6, respectively.

Thus, it would seem that cooking methods as different as the old-fashioned and modern methods did not have any greater effect on the calcium, phosphorus, ascorbic acid, and carotene contents of cooked beans than the daily market variation. These findings emphasize the importance of research on best methods of marketing vegetables for control of nutritive value and quality. The results would also indicate that the disagreement among reports on the effect of cooking method is due to the fact that this variant is not as important as many other variables in determining the nutritive value of vegetables.

SUMMARY AND CONCLUSIONS

Green beans, purchased from a local market on nine different days, were cooked in an open kettle with a sufficient amount of water to cover the vegetable, in a tightly covered kettle with a minimum of water, by the modified old-fashioned method and by the old-fashioned method. Both Frenched and snapped beans were cooked by the first two methods. The analysis of variance was used to study the data.

Basing the conclusions on the amounts in the total lots, there were no significant differences in the calcium content of beans either raw or cooked by the various methods. Phosphorus losses were not severe and in only one case, Frenched beans cooked in the open kettle, was there a significant loss. Carotene was quite stable during cooking since a significant amount was lost only in the case of snapped beans cooked in a tightly covered kettle. The primary loss during cooking of beans was in ascorbic acid and the percentage retained varied from 61.6 in the snapped beans cooked in a tightly covered kettle to 16.7 for those cooked by the old-fashioned method. Frenched beans, although scored higher for palatability than snapped beans, did suffer greater ascorbic acid losses.

Although these significant losses were found in beans owing to cooking, the analysis of variance showed clearly that as far as the calcium, phosphorus, ascorbic acid, and carotene contents of snap beans were concerned, the daily market variation (replication) had as much effect as cooking method. The importance of further research on marketing of fresh vegetables for preservation of nutrients was brought out.

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VARIABILITY OF ASCORBIC ACID WITHIN AND BETWEEN CABBAGES

ELIZABETH B. SMITH, MARY C. HILTZ, AND ALLEN D. ROBINSON

Departments of Foods and Nutrition and of Chemistry, University of Manitoba, Winnipeg, Canada

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A review of the literature on the ascorbic acid content of cabbage reveals such wide variations that it is difficult to state with any degree of confidence what figure or even what range of figures should be used to indicate the vitamin C value. In this paper we hope to show a method for obtaining data that can be treated statistically to obtain a mean that is the best representation of the ascorbic acid value of cabbage.

In a compilation of vitamin values by Booher, Hortzler, and Hewston (1942) the lowest figure for the vitamin C content of raw white cabbage is 14.3 mg. per 100 gm. and the highest figure is 180.9 mg. per 100 gm. This great variability is due to many factors among which variety and season are undoubtedly important. These two factors were stressed particularly by the following investigators: Gould, Tressler, and King (1936), working with six varieties, three summer and three winter, state that summer cabbage has more vitamin C than winter cabbage. Burrell, Brown, and Ebright (1940), using 30 varieties and strains, report a range from 48 to 180.9 mg. per 100 gm. with the higher values for summer cabbage. Lampitt, Baker, and Parkinson (1945) analyzed cabbages at various times throughout the year. In all they had 334 analyses with an average vitamin C content of 0.6 mg. per gm. for most of the year but with higher values for spring and early summer.

Not only is there a difference in the vitamin C value of cabbages owing to variety and season but the distribution of the vitamin is not uniform within the cabbage itself, as shown by the results of the following investigation: Sheets, Leonard, and Geiger (1941) reported that the outer green leaf blades of cabbage contained more vitamin C than the inner, bleached ones. Gallott (1942) also reported that the outer leaves of Milan cabbage, with a range from 130 to 140 mg. per cent, contained more vitamin C than did the inner leaves. On the other hand, Ranganathan (1935) obtained more for fresh inner leaves than for outer leaves with values of 132.6 and 125.8 mg. per cent, respectively. Last year in a preliminary investigation in our own laboratory it was found that the ascorbic acid concentration was highest in the core, being 88.1 mg. per cent. The content of the outer tissue was 48.5 mg. per cent, while the inner tissue was slightly higher at 52.2 mg. per cent. Further analysis this year showed that the outer quarter of local cabbage contained 31.3 mg. per cent, while the inner quarter, not including the core, contained 42.4 mg. per cent. Although these data do not agree as to the part of the cabbage which

contains the most vitamin, they are alike in confirming the lack of uniformity in the distribution of vitamin C within the plant.

In setting up the experimental procedure it is important to eliminate as many variable factors as possible and to choose a sampling method that will take into account the variation from cabbage to cabbage as well as the uneven distribution of the vitamin in the individual cabbage.

EXPERIMENTAL PROCEDURE

The cabbages to be analyzed for their vitamin C content were chosen from three different market gardens near the city of Winnipeg. The first garden cultivated the Penn. State Ballhead and the other two the Danish Ballhead. Ten cabbages were chosen at random from each of these three plots for the fresh analyses. The first 10 were of the Penn. State variety obtained from farm No. 1 in St. Vital, harvested Sept. 4. The second 10 were the Danish Ballhead variety from farm No. 2 in Headingly, harvested Sept. 24. The third lot of 10 were also the Danish Ballhead variety from farm No. 3 in St. Vital, harvested Oct. 8. On the day of harvesting they were stored in a refrigerator in the Old Science Building of the University of Manitoba at a temperature of 8 to 10°C.(46.4 to 50°F.). One cabbage was assayed daily over a period of 10 days immediately after each lot was harvested.

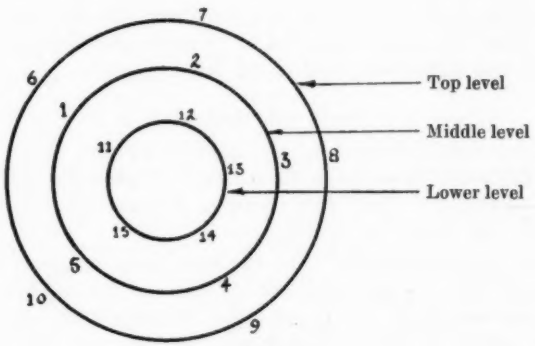

FIG. 1.

Since vitamin C distribution within the cabbage is not uniform, it was necessary to plan a method of sampling that would be representative of the different parts of the cabbage. This was done by making a special cutter, similar to an apple corer, so that each sample removed would be a cross section from the outer to the inner leaves. This special device was made of steel, saw-toothed at the cutting edge and fitted with a handle to help in withdrawing it. A wooden plunger helped to expel the sample from the corer. The size was such that when a sample was taken a weight of at least 25 grams was obtained. Since ascorbic acid in contact with certain metals is known to be highly oxidizable, the corer was electroplated with chromium.

Fifteen samples were taken from each cabbage, five in the top level, five in the middle level, and five in the bottom level. The five samples in each level were taken in a clockwise direction around the cabbage. Samples 1 to 5 were taken from the middle level, Samples 6 to 10 from the upper level, and Samples 11 to 15 from the bottom level. These samples were taken in such a way that the first samples of each level were directly in line with each other and were representative of one sector of the cabbage. The other samples were likewise taken in the same way. Each cabbage was thus divided into three levels and five sectors as shown (Fig. 1).

Each sample was weighed immediately, placed in the stabilizing acid at once, and assayed promptly. Duplicate analyses were made by the method of Loeffler and Ponting (1942), the readings being made on a Coleman spectrophotometer. As suggested by Morell (1941) a standard curve was prepared showing the concentration of synthetic ascorbic acid in milligrams per 100 milliliters. Results were interpreted by comparing the reading of the sample with the curve and using the equation given below:

$$\text{Conc. of sample} = \frac{\mu\text{g. ascorbic acid} \times 100 \times 350 \dfrac{(\%\text{ moisture of } 25)}{100}}{1{,}000 \times 25}$$

Moisture determinations were made on each of the cabbages of the third series from farm No. 3. As the moisture did not vary more than 1.5 per cent from the value suggested by Winton and Winton (1935), e.g., 91.5 per cent, this figure was used in all calculations.

RESULTS AND DISCUSSION

The ascorbic acid contents of the cabbages taken from farm No. 1 are listed (Table 1), those from farm No. 2 (Table 2), and those from farm No. 3 (Table 3). They are arranged so that the levels are shown vertically and the sectors horizontally.

Examining the data (Table 1) by the analysis of variance, as described by Goulden (1939), we find that the F value of the cabbages, which is 36.34, is higher than the five-per cent point, which is 1.99; also the F value of the levels, which is 1.92, is higher than the five-per cent point, which is 1.70; and the F value of the sectors, which is 0.15, is lower than the five-per cent point, which is 1.61. This shows that the differences in the ascorbic acid contents of the 10 cabbages and the levels are significant while the differences between the sectors are not significant.

Confidence intervals for the mean and the standard deviation cannot be computed since the distribution of the ascorbic acid content of the cabbages from this farm was nowhere near normal.

The steady increase in the mean value of the ascorbic acid contents of the cabbages during the 10-day period is surprising. It may be due to the relative immaturity of this lot of cabbages which was harvested on Sept. 4, somewhat earlier than is the custom for winter cabbages.

Examining the data (Table 2) by the analysis of variance we find that the F value of the cabbages, which is 71.76, is higher than the five-

TABLE 1

Ascorbic Acid Contents of 10 Cabbages (Penn. State Ballhead) From Farm No. 1

Levels (mg./100 gm.)			Levels (mg./100 gm.)		
Sector (1)			Sector (2)		
1	42.5	45.4	41.7	43.9	41.7	41.7
2	44.4	36.5	42.9	43.9	42.5	45.0
3	41.7	44.4	42.5	42.9	42.9	42.9
4	41.7	41.7	42.5	44.4	43.9	42.9
5	42.5	44.4	43.9	42.5	44.4	41.7
Mean—42.6. Standard deviation—1.99				Mean—43.1. Standard deviation—2.20		
Range—36.5-45.4				Range—41.7-45.0		
Weight (gm.)—1,089				Weight (gm.)—1,175		
Sector (3)			Sector (4)		
1	66.3	62.6	62.6	64.1	63.3	63.3
2	63.3	62.6	61.8	62.6	64.8	63.3
3	61.8	63.3	61.1	61.1	66.8	63.3
4	62.6	64.8	63.3	64.1	62.6	61.8
5	63.3	62.6	62.6	63.3	61.8	62.6
Mean—62.9. Standard deviation—2.17				Mean—62.9. Standard deviation—1.87		
Range—61.1-66.3				Range—61.1-64.8		
Weight (gm.)—1,010				Weight (gm.)—1,740		
Sector (5)			Sector (6)		
1	64.8	64.1	63.3	63.9	65.8	66.0
2	63.3	64.1	61.1	64.8	66.3	64.4
3	61.8	64.8	64.1	63.3	61.5	63.3
4	63.3	63.3	61.5	63.3	63.7	63.3
5	64.8	62.6	63.3	66.3	61.5	66.3
Mean—63.3. Standard deviation—2.05				Mean—64.2. Standard deviation—2.90		
Range—61.1-64.8				Range—61.5-66.3		
Weight (gm.)—1,540				Weight (gm.)—1,951		
Sector (7)			Sector (8)		
1	63.9	64.4	63.3	68.5	68.5	65.2
2	64.8	64.1	63.7	67.3	69.3	64.8
3	66.3	64.8	63.7	69.3	67.0	69.3
4	61.8	63.7	64.8	67.3	67.0	68.5
5	63.3	61.8	64.1	67.0	66.3	68.5
Mean—63.9. Standard deviation—1.10				Mean—67.5. Standard deviation—3.66		
Range—61.8-66.3				Range—65.2-69.3		
Weight (gm.)—957				Weight (gm.)—1,155		
Sector (9)			Sector (10)		
1	66.3	65.5	65.8	69.3	68.8	71.5
2	66.3	65.8	65.2	70.8	69.3	69.3
3	65.5	67.8	65.5	69.3	69.3	71.5
4	64.8	66.3	66.3	71.5	69.3	69.3
5	66.3	66.3	66.3	68.8	69.3	68.5
Mean—66.0. Standard deviation—0.67				Mean—69.7. Standard deviation—1.95		
Range—64.8-67.8				Range—68.5-71.5		
Weight (gm.)—840				Weight (gm.)—1,075		

TABLE 2

Ascorbic Acid Contents of 10 Cabbages (Danish Ballhead) From Farm No. 2

Levels (mg./100 gm.)			
Sector	**(1)**		
1	50.7	49.5	55.9
2	50.7	49.9	54.8
3	50.7	55.4	52.1
4	52.1	48.4	62.6
5	51.8	49.9	62.9

Mean—53.2. Standard deviation—4.30
Range—48.4-62.9
Weight (gm.)—2,303

Levels (mg./100 gm.)			
Sector	**(2)**		
1	52.1	47.7	54.8
2	52.1	49.5	52.1
3	49.5	48.9	53.3
4	46.9	49.9	53.9
5	52.1	50.7	50.7

Mean—50.9. Standard deviation—3.08
Range—46.9-54.8
Weight (gm.)—2,553

Sector	**(3)**		
1	49.5	49.9	51.4
2	51.4	48.4	52.1
3	56.6	49.5	57.8
4	52.1	48.0	58.8
5	59.1	52.9	62.9

Mean—53.3. Standard deviation—4.40
Range—48.0-62.9
Weight (gm.)—2,807

Sector	**(4)**		
1	68.5	68.5	72.7
2	72.7	69.3	71.5
3	68.8	68.8	73.7
4	63.0	64.5	68.5
5	67.8	67.8	67.8

Mean—68.9. Standard deviation—2.08
Range—67.8-73.7
Weight (gm.)—2,493

Sector	**(5)**		
1	59.6	56.6	61.8
2	53.6	60.3	55.9
3	59.6	61.1	58.2
4	64.8	64.8	61.8
5	52.9	59.6	55.9

Mean—59.1. Standard deviation—3.48
Range—52.9-64.8
Weight (gm.)—2,048

Sector	**(6)**		
1	50.4	49.2	50.7
2	50.7	52.4	50.7
3	48.4	49.5	51.4
4	53.3	52.9	52.1
5	50.4	52.1	50.4

Mean—50.9. Standard deviation—1.48
Range—48.4-53.3
Weight (gm.)—1,678

Sector	**(7)**		
1	47.7	49.5	52.9
2	52.1	47.7	52.9
3	55.4	47.7	53.9
4	46.9	45.9	50.4
5	49.5	47.7	50.4

Mean—50.4. Standard deviation—2.76
Range—45.4-55.4
Weight (gm.)—2,102

Sector	**(8)**		
1	48.4	54.8	48.9
2	48.4	52.1	47.4
3	51.4	54.8	47.4
4	49.5	53.9	47.4
5	49.5	51.4	47.4

Mean—50.2. Standard deviation—2.22
Range—47.4-54.8
Weight (gm.)—2,123

Sector	**(9)**		
1	49.5	54.4	52.1
2	49.2	53.9	52.1
3	49.5	49.5	50.7
4	49.5	49.5	52.9
5	56.6	49.5	52.1

Mean—51.4. Standard deviation—2.22
Range—49.2-56.6
Weight (gm.)—2,668

Sector	**(10)**		
1	67.0	66.3	61.1
2	67.0	63.7	61.8
3	64.8	63.7	64.1
4	67.8	61.1	60.3
5	61.4	61.4	63.7

Mean—63.6. Standard deviation—0.59
Range—60.3-67.8
Weigt (gm.)—1,290

per cent point, which is 1.99; also the F value of the levels, which is 3.14, is higher than the five-per cent point, which is 1.92; and the F value of the sectors, which is 2.33, is higher than the five-per cent point, which is 1.61. This shows that the differences in the ascorbic acid contents of the 10 cabbages, the levels, and the sectors are all significant.

The 95-per cent confidence interval for the mean value of the ascorbic acid content of the cabbages from farm No. 2 is from 54.08 to 56.36. The 95-per cent confidence interval for the standard deviation of the results is from 2.74 to 3.04.

Examining the data (Table 3) by the analysis of variance we find that the F value of the cabbages, which is 8.28, is higher than the five-per cent point which is 1.99; also the F value of the levels, which is 2.05, is higher than the five-per cent point which is 1.70; and the F value of the sectors, which is 0.84, is lower than the five-per cent point, which is 1.61. This shows that the differences in the ascorbic acid contents of the 10 cabbages and the levels are significant while the differences between the sectors are not significant.

The 95-per cent confidence interval for the mean value of the ascorbic acid content of cabbages from farm No. 3, is from 62.77 to 63.63. The 95-per cent confidence interval for the standard deviation of the results in this case is from 1.81 to 2.57.

If one could observe all possible samples in the cases of farms No. 2 and 3, 95 per cent of the time we would obtain values for the mean and standard deviation which would lie in the above intervals for the respective farms.

SUMMARY

An individual corer was devised which takes accurate samples of the cabbage so that when 15 samples are analyzed the mean gives the best representation of the ascorbic acid content of that individual cabbage. The range of values of the ascorbic acid content of 30 cabbages representing three market gardens near Winnipeg was from 36.5 to 73.7 mg. per 100 gm. The variability in cabbages and between cabbages is such that only analyses of all parts of the cabbage and many cabbages can give a mean that is the best picture of the vitamin C content.

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TABLE 3

Ascorbic Acid Contents of 10 Cabbages (Danish Ballhead) From Farm No. 3

Levels (mg./100 gm.)			Levels (mg./100 gm.)		
Sector (1)			Sector (2)		
1	55.4	55.4	57.8	66.7	67.8	68.5
2	55.9	56.6	55.4	68.5	67.0	67.0
3	58.4	54.4	56.6	66.3	65.6	67.0
4	55.4	57.8	54.4	65.6	66.7	66.7
5	53.6	50.6	53.6	66.3	65.6	66.3
Mean—55.4. Standard deviation—1.76				Mean—66.7. Standard deviation—3.25		
Range—50.6-58.4				Range—65.6-68.5		
Weight (gm.)—1,610				Weight (gm.)—1,315		
Sector (3)			Sector (4)		
1	63.7	64.1	65.3	58.8	60.3	59.3
2	63.7	64.1	62.6	57.8	64.1	64.1
3	61.1	59.3	52.4	61.1	60.3	64.8
4	61.8	61.1	61.4	64.8	61.8	64.8
5	62.6	59.6	62.9	59.9	60.3	59.9
Mean—62.4. Standard deviation—2.05				Mean—61.5. Standard deviation—1.47		
Range—59.3-65.3				Range—57.8-64.8		
Weight (gm.)—1,502				Weight (gm.)—1,836		
Sector (5)			Sector (6)		
1	63.7	64.8	64.8	70.0	69.3	72.3
2	67.0	69.3	62.6	69.7	71.2	67.3
3	67.8	66.3	67.0	70.8	70.0	69.3
4	66.3	66.7	67.8	73.0	71.5	69.3
5	66.3	63.7	64.8	73.0	70.0	69.3
Mean—65.9. Standard deviation—2.06				Mean—70.4. Standard deviation—1.52		
Range—62.6-69.3				Range—67.3-73.0		
Weight (gm.)—1,330				Weight (gm.)—1,735		
Sector (7)			Sector (8)		
1	62.9	61.8	64.1	67.0	64.1	64.1
2	62.9	61.8	59.6	66.7	64.1	66.3
3	58.8	63.7	59.6	71.8	64.8	65.3
4	63.7	62.3	60.3	66.7	66.3	66.7
5	62.9	62.9	61.8	66.7	65.3	66.3
Mean—61.9. Standard deviation—2.74				Mean—66.1. Standard deviation—1.69		
Range—58.8-64.1				Range—64.1-71.8		
Weight (gm.)—1,788				Weight (gm.)—1,386		
Sector (9)			Sector (10)		
1	61.1	58.4	57.8	64.8	62.3	61.4
2	56.6	58.8	57.4	62.6	61.4	62.3
3	55.9	60.3	56.6	63.7	61.4	63.7
4	56.9	59.6	59.6	64.1	62.3	63.7
5	57.8	57.4	58.1	61.8	62.9	62.6
Mean—58.1. Standard deviation—2.60				Mean—62.7. Standard deviation—2.03		
Range—55.9-61.1				Range—61.4-64.8		
Weight (gm.)—1,142				Weight (gm.)—1,112		

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EFFECT OF STORAGE TEMPERATURE ON RETENTION OF ASCORBIC ACID IN ORANGE JUICE

WM. EVENDEN AND GEO. L. MARSH
Food Technology Division, University of California, Berkeley, California

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Commercial distribution of orange juice by bulk shipments under refrigeration from the producing areas to consumption centers has received considerable thought. Essentially such schemes plan to locate the juicing plant within the producing areas. Then by utilizing the best possible extraction procedure, it is planned to thoroughly deaerate and quickly refrigerate the juice to a low temperature. The refrigeration would be carried out either at the plant or while en route by truck to the distribution area. Several points of controversy concerning the transportation phases of the procedure exist but they have no bearing on this discussion. At the distribution center a licensed plant would break down the bulk shipment to both retail and institutional packages and take care of its distribution thereafter.

Whenever such plans are discussed, the question always arises as to the stability of ascorbic acid under such treatment. Since in most cases the desire is to avoid a heat treatment in the process, largely because of its undesirable effect upon flavor and its cost, the question of the effect of preservatives on the stability of ascorbic acid also arises. Under the planned system preservatives almost certainly would be required to prevent the microbial count from unduly increasing during the distribution of the juice. Even when packed under the most sanitary conditions, the juice will contain an appreciable number of microorganisms which should not be allowed to increase appreciably.

The public generally and some scientific workers, Floyd and Fraps (1942), still believe that orange juice loses its ascorbic acid rapidly after extraction. Statements appearing in the press, based upon the work of Daniel, Kennedy, and Munsell (1936), Kohman (1924), and Hess and Unger (1919), to the effect that orange juice should not be squeezed and stored in the refrigerator the night before use are probably most responsible for the present beliefs.

Such statements, however, are not supported by scientific evidence developed since chemical methods were made available for determining ascorbic acid. Early workers—Joslyn, Marsh, and Morgan (1934) and McElroy, Munsell, and Steinbarger (1939)—showed the comparative stability of ascorbic acid in orange juice as compared with the older notions. Recent workers—Rohrer and Treadwell (1944), Scanlan and Willard (1944), and Smith, Ross, and Caldwell (1944)—continue to substantiate these findings.

An extensive literature has developed on the effect of many factors on ascorbic acid retention in citrus juices. Most of these works, however, pertain to studies on the climatic and cultural factors during maturation, on

methods of processing, on changes occurring during storage of the fresh and processed juice, and the like. Studies on the effect of temperature on the rate of oxidation of ascorbic acid in orange juice have not appeared in the literature to date. The present study was, therefore, undertaken to furnish this information.

Since rate of oxidation itself, at any given temperature, depends among other things upon the oxygen tension to which the juice is exposed, it also becomes a factor in this study. In order to obtain data that would be applicable to the limits of good and poor commercial handling practice, the tests were designed to obtain the rate of oxidation under a limited and under an ample supply of oxygen. This was accomplished by determining the rate of oxidation on deaerated and non-deaerated samples. In the deaerated sample, oxygen was not a limiting factor in the strictest sense, as sufficient head space was provided in the containers to supply the amount needed for oxidation of the ascorbic acid present in the juice. Deaeration simply made oxygen less readily available for reaction by removing the greatest percentage of the dissolved air.

EXPERIMENTAL PROCEDURE

Navel oranges from the Citrus Experiment Station, Riverside, were used in this series of tests. The fruit was fresh and in good condition and had not undergone the severe handling which much of the fruit now going into products receives. It was obtained in two shipments, one in early February, the other in mid-March. The first received in February was used for preparation of the deaerated series, while the fruit received in March was used for preparation of the non-deaerated series of samples.

Extra care was taken to avoid corrodible metal contacts during extraction of the juice. The lots of juice for the deaerated and non-deaerated series were prepared in their entirety as rapidly as possible before further breakdown into subseries. The fruit was cut with a stainless-steel knife and extracted with a Blessing extractor (burring unit) made of aluminum. Coarse pulp and much of the other suspended matter was removed by screening through an aluminum plate containing 80 holes of one millimeter diameter per square inch. Thereafter the juice was only in contact with glass which had been chemically cleaned and thoroughly rinsed with distilled water.

Deaeration of the juice, for the deaerated series of samples, was accomplished by holding the entire bulk of the juice under the highest vacuum obtainable with a water aspirator pump. This was a vacuum slightly in excess of 28.5 inches. During the 15-minute deaeration treatment the bottle was shaken several times to aid removal of the dissolved gases from the juice. At the end of this period the vacuum was relieved with N_2 and the juice was delivered into separate containers by N_2 pressure. The air in each container had been previously displaced by N_2 and it also contained a weighed amount of preservatives. After thorough stirring to dissolve the preservative the juice was driven by N_2 under pressure into six-ounce juice bottles which were sealed with crown caps. The control sample was treated in an identical manner except for addition of preservatives.

Approximately the same amount of juice was delivered to each bottle, 120 ± 1 ml. into a bottle of 190 ml. capacity. This amount of juice gave

the minimum head space required of this container for freezing storage. Sets of bottles consisting of all treatments were then stored at temperatures of approximately —16°C.(3°F.); 0°C.(32°F.; 10°C.(50°F.); and 25°C.(77°F.) for periodic determination of vitamin C content.

The non-deaerated series of samples was treated similarly in all respects, with the exception of those treatments relating directly to deaeration and transfer of the juice under N_2 gas pressure.

Four series of samples, relating to kind of chemical preservative used, were prepared with deaerated juice. They consisted of the following: (a) control, no preservative added; (b) 1,000 p.p.m. sodium benzoate added; (c) 500 p.p.m. monochloracetic acid added; and (d) 500 p.p.m. sodium benzoate, 500 p.p.m. sulfur dioxide. Five series were prepared with the non-deaerated juice. They likewise related to kind of chemical preservative used and were as follows: (a) control, no preservative added; (b) 1,000 p.p.m. sodium benzoate; (c) 500 p.p.m. monochloracetic acid; (d) 500 p.p.m. sodium benzoate, 500 p.p.m. sulfur dioxide; and (e) 500 p.p.m. sulfur dioxide.

The juice used for setting up the two series of experiments differed somewhat in their gross chemical compositions and flavor characteristics but were quite similar with respect to their vitamin contents. The juice for the deaerated series was 13.1° Brix (20°C.), contained 1.41 gm. acid per 100 ml. (as anhydrous citric acid), and contained 70 mgm. of ascorbic acid per 100 ml. The Brix-to-acid ratio was therefore 9.3, and it had a pH of 3.31. That used for the non-deaerated series, tested 13.3° Brix (20°C.), contained 1.035 gm. acid per 100 ml., which was a Brix-to-acid ratio of 12.9. The ascorbic content of this juice was 68 mgm. per 100 ml.

The ascorbic acid content of the storage samples was determined at periodic intervals by both a 2.6-dichlorophenolindophenol titration and an iodine titration. The iodine solution was standardized against thiosulfate which had previously been standardized against potassium dichromate by the Hodgeman and Lange procedure. The 2.6 dichlorophenolindophenol was prepared and standardized by the Menaker and Guerrant (1938) method, slightly modified. In both determinations five ml. of orange juice diluted with 50 ml. of distilled water in 125-ml. Erlenmeyer flasks was found to give the most definite and reproducible end points. The determinations were conducted on triplicate amounts of the same sample. The juice was titrated with ca. 0.005N dye to a faint permanent pink end point, and to the starch end point with 0.01N iodine solution.

A single bottle of each treatment was removed from storage at each testing interval. All were brought to ca. 20°C. in running water before conducting the ascorbic acid determinations.

RESULTS OF RATE DETERMINATIONS

The composite results of this investigation are reported (Table 1); the values reported are the percentage of the original ascorbic acid content remaining in the sample at time of testing. Only those values obtained by the dye titration are reported in the table for two reasons: first, the dye titration is generally recognized as yielding the more acceptable ascorbic acid values; and second, since there was no very essential difference

between the results of the two methods reporting, the iodine values would be repetitious. The chief difference noted was that the ascorbic acid content calculated from iodine values was slightly higher than those calculated from dye titrations, i.e., 72 and 70 mgm. ascorbic acid per 100 ml., respectively, for the original juice. However, this condition also holds with a fair degree of constancy on the stored samples, and the percentage retention values are nearly identical in all cases.

One other reason for reporting only the dye values is that iodine values obtained in the presence of added reducing substances are meaningless without supplementary analytical information. Sulfites have been shown to cause no interference in the direct titration of citrus juice by the dye solution, Hamburger and Joslyn (1941), if the solution is acid and the titration is carried out rapidly. This was likewise found to be the case during this investigation.

DISCUSSION

An examination of Table 1 shows that this investigation has brought out a number of facts worthy of note, some of which are apparent and others only interpretable by aid of additional facts. Of primary importance, perhaps, is the fact that orange juice retains large percentages of its ascorbic acid content in the reduced state for significantly long periods at all temperatures below room temperature in the presence of more oxygen than is required for its oxidation. Oxidation of ascorbic acid in orange juice by atmospheric oxygen is a relatively slow phenomenon at all temperatures lower than ordinary room temperature under non-agitating conditions. The presence of the permissible preservative, sodium benzoate, or the nonpermissible monochloracetic acid, have no detrimental effect on the retention of ascorbic acid in orange juice. The presence of sulfur dioxide as a preservative against microbiological spoilage inhibits the oxidation of ascorbic acid, now a fact generally recognized.

Examination of the table would indicate that both sodium benzoate and monochloracetic acid accelerate the oxidation of ascorbic acid at temperatures above freezing, when the results of the control samples and the latter two are compared. This is not the case, however, as orange juice not containing microbial spoilage inhibitors quickly undergoes fermentation. With onset of fermentation, oxygen, either dissolved and/or atmospheric, is quickly utilized by the microorganisms involved, and further loss of ascorbic acid by direct oxidation is thereby prevented. An accompanying drop in redox potential is also an assisting mechanism in preventing further loss of ascorbic acid. Fermentation occurred in the deaerated control samples stored at 25°C.(77°F.) in the majority of the samples before the seventh day, in those stored at 10°C.(50°F.) before the 13th day, and in those stored at 0°C.(32°F.) before the 34th day. Approximately the same events occurred in the non-deaerated controls, but because of their greater original oxygen supply the samples dropped to a lower ascorbic acid content before the oxygen was completely utilized by the microorganisms than did the deaerated controls.

Monochloracetic acid would appear to be less destructive to ascorbic acid in orange juice than sodium benzoate, on prolonged storage tests at temperatures of 25 and 10°C. Again, however, this is not the actual case,

TABLE 1

Retention of Ascorbic Acid in Orange Juice Under Various Treatments

Treatment	3 days		7 days		13 days		34 days		67 days		96 days		130 days		163 days	
	D[1]	U[1]	D	U	D	U	D	U	D	U	D	U	D	U	D	U
Stored 0-5°F.	*pct.*		*pct.*		*pct.*		*pct.*		*pct.*		*pct.*		*pct.*		*pct.*	
1. Control	....	....	....	....	....	....	91	100	....	80	94	80	91	....	93	....
2. Sodium benzoate	....	....	....	....	....	....	94	94	....	92	99	85	93	....	94	....
3. Monochloracetic acid	....	....	....	....	....	....	93	100	....	93	99	90	89	....	94	....
4. Sodium benzoate and sulfur dioxide	....	....	....	....	....	....	93	100	....	90	99	87	90	....	94	....
5. Sulfur dioxide	....	....	....	....	....	....	....	100	....	81	....	90	....	....	....	....
Stored 32-34°F.																
1. Control	....	....	....	....	....	....	90	74	....	64	84	57	84	....	84	....
2. Na B_s—1,000 p.p.m.	....	....	....	....	....	....	84	75	....	57	60	52	47	....	51	....
3. CH_2 Cl COOH—500 p.p.m.	....	....	....	....	....	....	81	75	....	60	61	50	49	....	43	....
4. Na B_s—500 p.p.m.	....	....	....	....	....	....	90	81	....	71	89	74	83	....	84	....
4. SO_2—500 p.p.m.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
5. SO_2—500 p.p.m.	....	....	....	....	....	....	....	85	....	76	....	78	....	....	....	....
Stored 50-54°F.																
1. Control	97	....	....	94	97	....	87	71	....	74	86	70	74	....	....	....
2. Na B_s—1,000 p.p.m.	97	....	....	91	90	....	66	54	....	30	47	16	19	....	....	....
3. CH_2 Cl COOH—500 p.p.m.	97	....	....	94	89	....	69	61	....	70	66	60	41	....	....	....
4. Na B_s—500 p.p.m.	99	....	....	97	97	....	87	76	....	66	77	59	70	....	....	....
4. SO_2—500 p.p.m.	99	....	....	97	97	....	87	76	....	66	77	59	70	....	....	....
5. SO_2—500 p.p.m.	....	....	....	100	....	....	....	78	....	68	....	62	....	....	....	....
Stored 75-80°F.																
1. Control	97	....	....	93	99	....	86	78	....	75	46	74	27	....	....	....
2. Na B_s—1,000 p.p.m.	94	....	....	79	79	....	29	31	....	6	23	6	20	....	....	....
3. CH_2 Cl COOH—500 p.p.m.	94	....	....	97	81	....	51	79	....	76	44	67	64	....	....	....
4. Na B_s—500 p.p.m.	99	....	....	91	94	....	74	63	....	41	44	31	33	....	....	....
4. SO_2—500 p.p.m.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
5. SO_2—500 p.p.m.	....	....	....	97	....	....	....	65	....	44	....	34	....	....	....	....

[1] D = deaerated; U = undeaerated.

as 500 p.p.m. of monochloracetic acid failed to prevent growth of spoilage organisms beyond 34 days in the deaerated samples and 13 days in the non-deaerated samples at these temperatures.

Samples containing sulfur dioxide alone and those containing sulfur dioxide in combination with sodium benzoate behave almost identically with respect to retention of ascorbic acid under these conditions. From the standpoint of prolonged storage at temperatures above freezing, the juice containing the two preservatives in combination would resist microbial spoilage for longer periods than that containing sulfur dioxide alone. The latter, however, would be the preferable treatment for juice undergoing short storage periods where flavor and ascorbic acid retention are the primary considerations. Half the amount used in these investigations would suffice for this purpose.

A word of explanation concerning the use of navel orange juice for this investigation is perhaps necessary. Since flavor was not a factor under study, the variety of orange used was immaterial. Previous work by Joslyn, Marsh, and Morgan (1934) had shown no essential difference between Valencia juice and navel juice in the loss of reducing value under oxidation by air. Both orange juices behave quite similarly in this regard. It is realized, however, that flavor is a factor of equal importance, perhaps, to ascorbic acid retention in the marketing of citrus juices. It is generally recognized by food technologists, primarily on the basis of long observation, that any treatment acting to prevent destruction of ascorbic acid in food products without itself actually destroying flavor also acts to prevent undue flavor changes within the product. A case in point is properly prepared and subsequently handled frozen foods. On the basis of these tests it is reasonably safe to predict that orange juice treated to remove dissolved air, handled to prevent an undue amount of re-aeration, and stored at or near 0°C. will retain an acceptable flavor for 15 days and perhaps slightly longer. This should be ample time to move large quantities of orange juice into consumption as liquid juice from centrally located juicing stations for both consumer and institutional use.

One other factor entering into any considerations of the use of low above-freezing temperature as an aid to marketing citrus juices remains to be discussed. Unless the pectic enzymes present in citrus juices are inactivated by heat, they bring about a clarification of the juice. The result is an unsightly floc that does not entirely re-suspend with shaking. Heat inactivation of the pectic enzymes as done at present, however, results in considerable damage to flavor. When it is not considered desirable to use heat because of this effect, the juice will have to be handled at the lowest possible temperature and quickly distributed for consumption. Juice going into freezing storage should be heat-treated for enzyme inactivation because of the likelihood of such juice being held for periods beyond that originally intended. In this investigation, juice held for only 96 days at —16°C.(3°F.) was completely flocculated upon thawing because of continued enzyme action at this temperature. All samples stored at 0°C. were clarified completely at the 34-day testing interval.

In addition to the more obvious facts already reported, critical examination of the data presented (Table 1) shows that the oxidation of ascorbic

[1] D = deaerated; U = undeaerated.

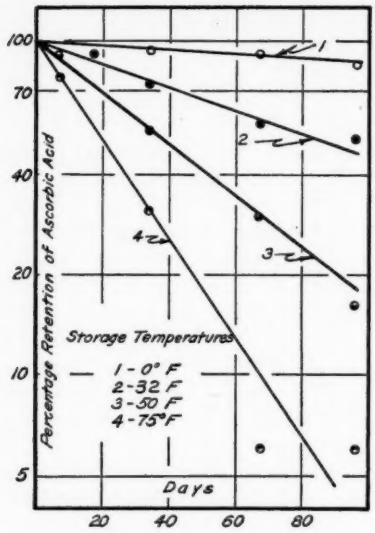

FIG. 1. Effect of temperature in retention of ascorbic acid in undeaerated orange juice preserved with sodium benzoate.

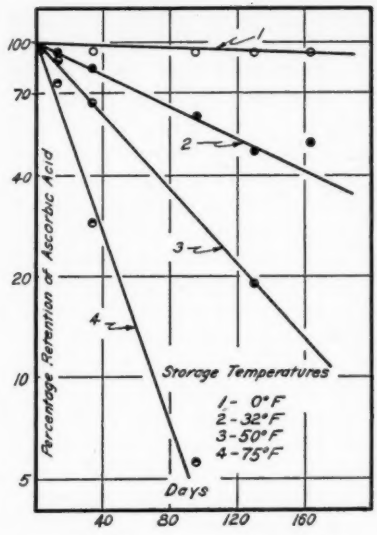

FIG. 2. Effect of temperature in retention of ascorbic acid in deaerated orange juice preserved with sodium benzoate.

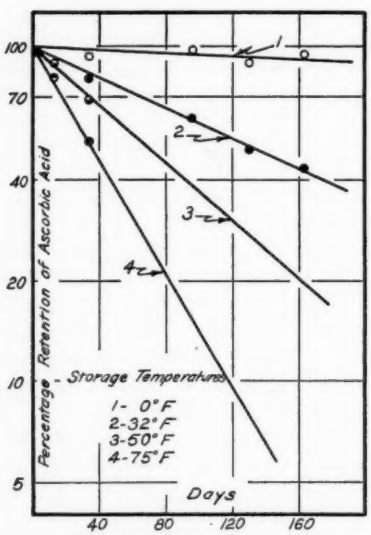

FIG. 3. Effect of temperature on retention of ascorbic acid in deaerated orange juice preserved with monochloracetic acid.

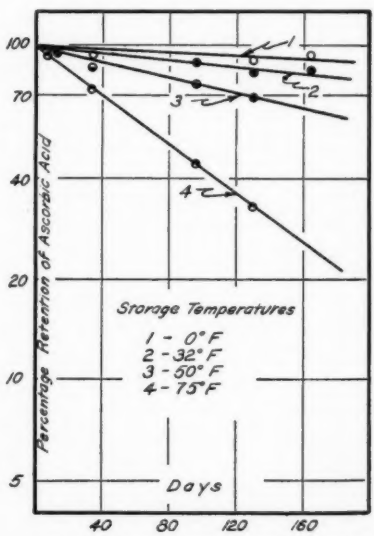

FIG. 4. Effect of temperature on retention of ascorbic acid in deaerated orange juice preserved with a combination of sodium benzoate-sulfur dioxide.

acid under the conditions of this experiment is essentially a first-order reaction. When the logarithm of the concentration, expressed as percentage of ascorbic acid retained in this case for simplicity, is plotted against time, straight lines are obtained for all storage temperatures. The addition of preservatives has no effect upon the order of the reaction as illustrated (Figs. 1 to 4).

Calculation of the specific reaction rate constant k (the fraction oxidized per day) from the data obtained for the deaerated juice preserved with sodium benzoate gives values of 0.00042, 0.00481, 0.01158, and 0.02872, for the temperatures —16, 0, 10, and 25°C., respectively. In other words, the rate of oxidation of ascorbic acid in orange juice preserved with sodium benzoate in the presence of just sufficient oxygen to complete the reaction is approximately 0.04, 0.5, 1.2, and 2.9 per cent per day for the temperatures above named, respectively. Extrapolation of the data obtained and calculation of k for temperatures of 30 and 40°C. show that the ascorbic acid oxidation at these temperatures would proceed at the rate of 4.0 and 7.1 per cent per day, respectively, provided no other factors entered into the reaction. The calculated half-life period for this treatment at the temperatures noted are 1,646, 144, 60, 24, 18, and 10 days, respectively.

A comparison of the various treatments as to their effect upon the rate of oxidation of ascorbic acid is best illustrated by the calculations presented (Table 2).

TABLE 2

Comparison of Various Treatments on Oxidation of Ascorbic Acid at 0° C.(32° F.)

Treatment	k X10^4	Half-life
		days
Deaerated juice preserved with sodium benzoate	48.1	144
Deaerated juice preserved with monochloracetic acid	52.0	133
Deaerated juice preserved with sodium benzoate and sulfur dioxide	11.3	614
Un-deaerated juice preserved with sodium benzoate	72.3	96

As is shown, no real significant difference exists between sodium benzoate and monochloracetic acid as to their effect on the rate of oxidation. This is true in spite of the fact that the latter has been claimed to exert antioxidant properties in addition to its preservative action against microorganisms. The addition of sulfur dioxide, on the other hand, does inhibit the rate of oxidation rather markedly. In the concentration used in this experiment it increased half-life time approximately fourfold. It should also be noted that deaeration, without further limitation on air supply, causes a 50-per cent increase in half-life time. Storing and handling deaerated juice in the absence of air would increase this very considerably. Since this would be comparable to storage of the juice in cans, the rate of oxidation likewise should be comparable to the canned product. Half-life times calculated from data presented by Ross (1944) for canned orange juice stored at 9 and 24°C. were 3,100 and 845 days, respectively.

The influence of temperature upon the rate of oxidation is illustrated (Fig. 5). Here, the relation between rate of oxidation and temperature is

tested by plotting the log of the rate against the reciprocal of the absolute temperature. Straight lines were obtained for each of the various treatments showing the data are in agreement with the Arrhenius equation. Since the slopes of these lines are practically identical it must be concluded, therefore, that the same reaction controls the rate at all temperatures. The apparent activation energy for the oxidation of ascorbic acid in orange juice as calculated from the slope of the rate-temperature curves, is of the order of 11,700 cals. The corresponding $Q_{10} = 2.25$.

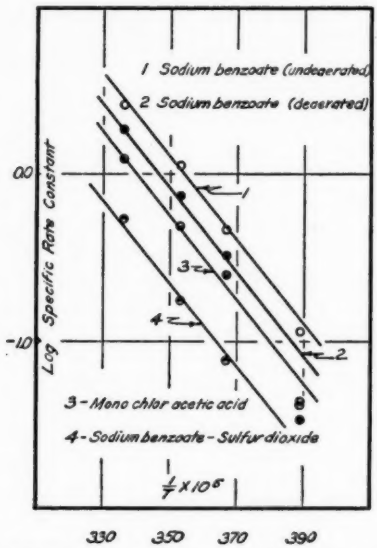

FIG. 5. Influence of temperature on rate of oxidation of ascorbic acid in orange juice preserved with various chemicals.

CONCLUSIONS

The oxidation of ascorbic acid in orange juice in the liquid state is a first-order reaction whose rate is a function of temperature. It is uncertain from the data obtained whether the same reaction controls the oxidation of ascorbic acid in the frozen-juice samples as in the unfrozen samples. The activation energy for the oxidation of ascorbic acid in orange juice is in the order of 11,700 cals.

The rate of oxidation of ascorbic acid in orange juice in the temperature range 10° C.(50° F.) to 0° C.(32° F.) is slow enough to permit its retail distribution through well-planned bulk handling procedures without appreciable loss of its ascorbic acid content. It is believed that undesirable flavor changes would serve as added insurance in this connection.

Sodium benzoate and monochloracetic acid have little effect on the rate of oxidation of ascorbic acid in orange juice. Sulfur dioxide lowers the rate of oxidation and would appear to be the best preservative to use either alone or in combination with sodium benzoate.

ACKNOWLEDGMENT

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SURVIVAL OF BACTERIA IN A PRECOOKED, FRESH-FROZEN FOOD[1]

M. F. GUNDERSON AND K. D. ROSE
Department of Pathology and Bacteriology, College of Medicine, University of Nebraska, Omaha, Nebraska

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There is a tendency among some producers of frozen foods to compromise sanitary production methods for those more traditional and peculiar to the industry, relying upon subsequent deep-freeze storage to reduce the bacterial count of their product. It is not difficult to understand how this tendency developed. The literature is replete with data on the destruction of bacteria by cold, Wallace and Tanner (1933) and Weiser and Osterud (1945). The error lies in the misapplication of this information. Smart (1934) discovered that as many as one million bacteria per gram of frozen food could remain viable in some instances after one year of storage, even though the average reduction in bacterial numbers was 99.3 per cent. McCleskey and Christopher (1941) reported that *Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi A, Salmonella paratyphi B,* and *Salmonella typhi* survived deep-freeze storage in unsliced strawberries after 14 months' storage at —18°C.(—0.4°F.). Thus, it is fallacious for a producer to assume that deep-freeze storage will "pasteurize" a contaminated product. Rosenau (1912), writing on the milk question, has said "pasteurization should never be used as a redemption process, cannot atone for filth, and cannot make good milk out of bad." The same might be said for frozen foods, wherein cold storage is used as a "pasteurization" process.

We became interested in the effect of cold storage on microorganisms during studies on the bacteriology of boned chicken production by Gunderson, Rose, and Henn (1947) and Rose and Gunderson (1947), wherein counts as high as 9,000,000 per gram were found in meat destined for inclusion in fresh-frozen chicken chow mein. Interest was heightened by the peculiar physical consistency of chow mein, the menstruum of which differs markedly from that of fruits and vegetables, which have been the subject of most studies of this nature—Berry (1933), Wallace and Park (1933), and McCleskey and Christopher (1941). Chicken chow mein is representative of a precooked food in which the menstruum contains an abundance of colloids, mostly in the form of emulsified fat. When frozen, it has a physical appearance resembling "snow ice" or "bubbly ice" as described by Keith (1913). It was assumed that the emulsified fat was responsible for this type of freezing, and further, that it might serve as

[1] The subject matter of this paper was undertaken in co-operation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the War Department.

protection for contained bacteria similar to its protective action in cream reported by Hilliard, Torossian, and Stone (1915). Thus, it seemed desirable to ascertain what effect cold storage might have on bacteria contained in chicken chow mein. In so doing we have attempted to answer to a certain extent the two fundamental questions propounded by Prescott and Tanner (1938), *viz.*, "What is the effect of low temperatures on pathogenic bacteria that might gain access to this food?" and "What is the effect on organisms of spoilage and on ordinary and relatively inactive saprophytes which are present in or upon the food material refrigerated?"

MATERIAL AND METHODS

The suspension medium employed in all these studies was commercially prepared chicken chow mein. This material contains a base of chicken meat, constituting 30 to 40 per cent of its solid bulk. In addition it contains chow mein noodles, bean sprouts, celery, onion, and a sauce made of chicken fat, chicken broth, and seasoning of various types. It has a pH of approximately 6.0 and, when subjected to a temperature of —25.5°C. (—14°F.), freezes to a mushy consistency. All of the material used was obtained in a freshly prepared and unfrozen state. When sterility was necessary, portions were autoclaved for one hour at 121°C.(249.8°F.). The change noted in its physical state following autoclaving consisted of slight browning, mushing, and a shift in pH to 5.8.

The eight strains of *Salmonella* employed were received originally from Dr. Paul Kabler of the Minnesota State Board of Health and are those used routinely in preparation of *Salmonella* typing sera. The cultures of *Escherichia coli* and *Aerobacter aerogenes* were stock strains from the University of Nebraska collection. For pure culture studies, one-half ml. of 16- to 20-hour broth cultures of these organisms were inoculated into 250 grams of sterile chicken chow mein and placed in small paper cartons. After original counts were made the cartons were cap-sealed, frozen, and stored at —14°F. Except where specifically noted, periodic sampling was carried out by shaking 1:10 dilutions of representative samples of frozen substrate in sterile, glass-stoppered, wide-mouth bottles, further dilutions and platings being made from them. "Total" counts were ascertained on tryptone glucose extract agar prepared according to standard methods. Difco violet red bile agar and blood agar base plus 0.5 per cent citrated blood were used in determining coliform and hemolytic *Staphylococcus* counts, respectively.

Studies by Smart (1939), Kiser and Beckwith (1942), and others seem to indicate that storage temperatures approaching 0°C.(32°F.) result in a greater decrease in bacterial numbers than that observed at lower temperatures. For our purposes, however, a temperature of —14°F. was chosen. This temperature corresponded with that of the cold-storage vaults in local processing plants. In addition, it falls within the range of temperatures of —12.5 to —35°C.(10 to —31°F.), in which all free water becomes frozen in a biocolloid, St. John (1931), and in which "storage death" occurs, Weiser and Osterud (1945). Freezing and cold storage was accurately maintained at —14°F. in a 10 x 24 x 9-inch Nash-Kelvinator metallurgical deep-freeze unit.

EXPERIMENTAL PROCEDURE

Experiment 1: Survival of Pure Cultures of Salmonella sp. in Chicken Chow Mein Stored at —25.5°C.(—14°F.): The change in bacteria count of artificially inoculated chicken chow mein during a storage period of nine months' duration (Table 1) shows that there was a rather rapid initial decline in bacterial numbers until the 14th day, 33 to 92 per cent being killed in that time. The subsequent decline was slow. Plotting these results (Fig. 1) gives a curve suggesting that the rate of death in the early

TABLE 1

Survival of Pure Cultures of Enteric Organisms in Chicken Chow Mein

Organism	Bacterial count per gram ($\times 10^8$) after storage (days)								
	0	2	5	9	14	28	50	92	270
S. newington	75.5	56.0	27.0	21.7	11.1	11.1	3.20	5.0	2.2
S. typhimurium	167.0	245.0	134.0	118.0	111.0	95.5	31.0	90.0	34.0
S. typhi	128.5	45.5	21.8	17.3	10.6	4.5	2.6	2.3	0.86
S. gallinarum	68.5	87.0	45.0	36.5	29.0	17.9	14.9	8.3	4.8
S. anatum	100.0	79.0	55.0	52.5	33.5	29.4	22.6	16.2	4.2
S. paratyphi B	230.0	205.0	118.0	93.0	92.0	42.8	24.3	38.8	19.0

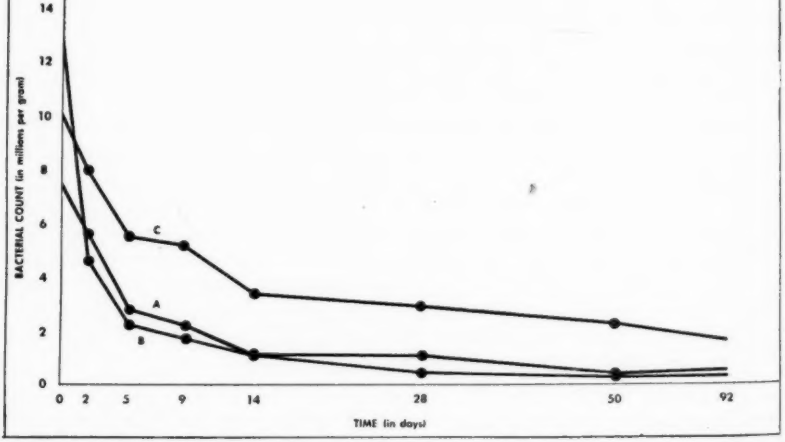

FIG. 1. The effect of storage in chicken chow mein at —14°F. on the survival of pathogenic enteric bacilli. A = *Salmonella newington.* B = *Salmonella typhi (Eberthella typhosa).* C = *Salmonella anatum.*

stages of storage is proportional to the number of viable bacteria present. After the 14th day a more or less stable population remains. Kiser (1943), working with *Achromobacter* species stored in sea water broth at —28°C. (—18.4°F.) and Tanner and Williamson (1928), working with yeast and bacteria frozen in broth and physiological saline at approximately 0°C.

(32°F.), obtained similar results. The fact that the physical consistency of these substrates differs greatly from chow mein suggests that colloid content of the suspension medium may not be an important factor in determining resistance of bacteria to freezing. However, Wallace and Park (1933), studying survival of *Salmonella typhimurium, Salmonella typhi,* and *Salmonella paratyphi B* in cherry juice, found that large inocula of these organisms were killed in two to three weeks by —17.8°C.(0°F.), whereas McCleskey and Christopher (1941), studying survival of the same organisms in sliced strawberries, found two to 14 per cent of the initial inocula survived four weeks at —18°C.(—0.4°F.) while 0.06 to one per cent survived eight months. In view of the fact that the temperatures were essentially the same and that pH of cherry juice and sliced strawberries is quite similar, these data indicate that physical consistency of the suspension medium may have been the important factor.

Attention is called to the 20 per cent nine months' survival of *Salmonella typhimurium,* a representative of the so-called "animal" *Salmonella.* These "animal" *Salmonella* species, although not responsible for a clinically specific disease such as paratyphoid fever, have frequently been involved in outbreaks of gastroenteritis. Should they prove to be relatively resistant to cold storage, their importance in frozen-food bacteriology would become enhanced.

TABLE 2

Survival of Natural Flora in Commercially Produced Chicken Chow Mein and Boned Chicken

Specimen	Number of samples	Medium[1]	Average counts ($\times 10^3$) after storage (days)					
			0	2	7	30	50	280
Chow mein	5	T.G.E.	TNTC	TNTC	85,600	64,200	48,300	25,750
		V.R.B.	21.8	11.0	6.6	1.1	0.5	0.48
Boned chicken	5	T.G.E.	3,850	9,930	2,790	2,750	.....	.....
		V.R.B.	32.2	0.125	0.520	0.380	.....	.....

[1] T.G.E. = tryptone glucose extract agar: V.R.B. = violet red bile agar (Difco).

Experiment 2: Survival of Normal Flora of Boned Chicken and Chicken Chow Mein: Data obtained by following the "total" bacteria count in boned chicken and chicken chow mein stored at —14°F. for periods ranging from 30 to 280 days are presented (Table 2). Thirty- to 40-gram samples of this material were obtained at intervals, thawed, and finely emulsified in 1:10 dilutions in a Waring blendor. Further dilutions and platings were made from the 1:10 dilution. The results show a rather sharp reduction in coliform flora but a slow decrease in total flora. Of particular importance is the exceptionally high bacterial numbers remaining in the commercial chicken chow mein even after nine months' storage. These are the bacteria responsible for spoilage in the thawed product, Gunderson, Rose, and Henn (1947). It serves to emphasize the need for keeping the initial contamination at a minimum.

Experiment 3: Comparison of Coliform Counts on Tryptone Glucose Extract Agar and Violet Red Bile Agar: The fact that selective media exhibit a certain degree of inhibition for the microorganisms they are

designed to isolate is not sufficiently appreciated. No bacterium is completely immune to the inhibitory action of any chemical or combination of chemicals. With this fact in mind, an experiment was designed to ascertain what effect violet red bile agar had on the determination of coliform numbers in cold-stored chicken chow mein. Two 250-gram specimens of chicken chow mein were inoculated with pure cultures of *Escherichia coli* and *Aerobacter aerogenes*. Initial counts (before freezing) and subsequent counts during storage at —14°F. were made simultaneously and from the same dilution bottles on tryptone glucose extract agar and on violet red bile agar; results are presented (Table 3). Assuming that 100 per cent

TABLE 3

Comparison of Coliform Counts on Tryptone Glucose Extract Agar and on Violet Red Bile Agar During Storage in Deep-Freeze at —14°F.

Organism	Medium[1]	Bacterial count ($\times 10^3$) after storage (days)								
		0	2	5	9	14	28	50	92	270
E. coli	T.G.E.	17,500	2,490	885	1,050	730	240	.....	212	13
	V.R.B.	8,510	955	164	275	121	77	77	31	8.3
	Per cent diff.[2]	48.6	38.3	18.5	26.2	16.6	32.1	.....	14.6	64.0
Aerobacter aerogenes	T.G.E.	14,950	8,100	2,300	2,145	1,680	1,280	635	570	132
	V.R.B.	8,750	3,730	610	630	520	300	169.0	.....	15.8
	Per cent diff.[2]	58.5	46.1	26.5	29.4	31.0	23.4	26.8	.....	12.0

[1] T.G.E. = tryptone glucose extract agar; V.R.B. = violet red bile agar (Difco). [2] The ratio V.R.B. to T.G.E.

of the viable microorganisms present at any given time grew on tryptone glucose extract agar, only 48.8 per cent of the *Escherichia coli* and 58.5 per cent of the *Aerobacter aerogenes* grew on violet red bile agar at zero time. The per cent able to grow on violet red bile decreased during cold storage until only approximately 25 per cent were able to grow after five days' storage. The ratio of growth of *Aerobacter aerogenes* on tryptone glucose extract vs. violet red bile agar is plotted against storage time (Fig. 2). A rapid increase in violet red bile inhibition is observed, becoming more or less constant after five days. By 270 days, 88 per cent of the viable organisms present were inhibited (Table 3). Cold storage, in some manner, renders viable coliforms less able to overcome the resistant action of the medium (cold injury). Although of great theoretical interest, to a food bacteriologist this phenomenon is more important from the practical standpoint. "Coliform" counts in cold-stored food, determined by selective medium cultivation may be only 12 to 25 per cent accurate. Any standard of bacterial purity must take this fact into consideration.

That this phenomenon may not be limited to *Escherichia coli* and *Aerobacter aerogenes* is shown (Fig. 3), which was taken from an experiment designed to ascertain survival of two enteric pathogens in chow mein containing its natural flora. Violet red bile was employed as the substrate and colorless colonies were counted. Apparently 91 per cent of the *Salmonella paratyphi A* and 96 per cent of the *Salmonella newport* were killed in 14 days. None was detected on the plates after 270 days'

storage. The 91 to 96 per cent 14-day death rate in this experiment is exceedingly rapid when compared with the 66 per cent average 14-day death rate in Experiment 1 (Table 1). However, it is in all probability an artifact. Without the information shown (Fig. 2), this artifact might have been erroneously interpreted. In light of these findings, existing data on the effect of cold storage on coliforms in fresh-frozen foods should be re-evaluated.

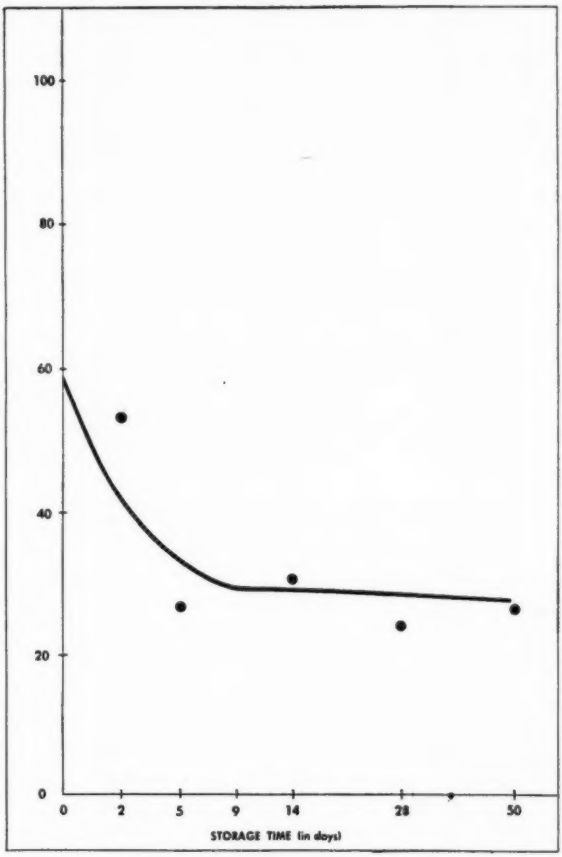

FIG. 2. Ratio of growth of *A. aerogenes* obtained on two media, $\frac{\text{V.R.B.}}{\text{T.G.E.}}$, during storage at —14°F. expressed in percentage.

Experiment 4: Numbers of Bacteria Occurring in Stored Products Containing Chicken: Representative samples of each day's commercial production of chow mein and chicken salad were examined for total, coliform, and hemolytic *Staphylococcus* numbers after storage at —12 to —14°F. for periods ranging from 46 to 157 days (Table 4). The median total count was 326,000 with extremes of 26,000 and 62,600,000 per gram. The

TABLE 4

Numbers of Viable Bacteria in Chicken Chow Mein and Chicken Salad Stored in Deep-Freeze for Periods Ranging From One and One-half to Five Months

Material	Storage time (days)	Total counts	Coliform counts	Hemolytic *Staphylococcus* counts
Chicken chow mein	157	116,000	5,200	0
	156	112,000	1,240	0
	156	760,000	21,800	0
	155	170,000	1,020	0
	138	640,000	920	200 [1]
	137	220,000	1,220	0
	136	326,000	6,800	0
	136	25,600,000	6,700	0
	135	2,380,000	12,000	2,000 [1]
	135	64,000	11,400	0
	133	660,000	2,890	0
	132	41,000	1,660	0
	131	40,000	2,900	0
	131	38,000	690	0
	130	36,000	740	100 [1]
	122	2,740,000	782,000	0
	121	560,000	0	100 [1]
	121	69,000	880	200 [1]
	120	10,000,000	158,000	0
	120	79,000	1,510	0
	119	2,960,000	0	2,000 [2]
	118	4,240,000	136,000	0
	117	153,000	1,260	0
	117	26,000	560	0
	116	194,000	10,600	0
	115	168,000	36,400	0
	115	88,000	3,940	0
	114	146,000	4,360	0
	106	6,700,000	29,400	0
	106	107,000	16,000	0
	105	48,000	1,450	0
	104	22,800,000	13,400	20,000 [2]
	104	1,800,000	4,420	0
	101	3,800,000	11,000	0
	99	3,020,000	6,200	0
	97	3,300,000	1,320	0
Chicken salad	62	620,000	2,380	14,000 [2]
	57	62,600,000	49,600	10,000 [2]
	56	1,340,000	33,800	2,000 [1]
	54	580,000	18,000	2,000 [2]
	53	660,000	9,800	20 [2]
	50	680,000	3,000	16,000 [2]
	46	258,000	1,720	20 [2]
	46	600,000	1,060	600 [1]

[1] Hemolytic *Staphylococcus albus* and/or non-hemolytic *Staphylococcus aureus* were isolated from these samples. [2] Hemolytic *Staphylococcus aureus* was isolated from these samples.

median coliform count was 3,000 with extremes of zero and 782,000 per gram. Attention is called to the fact that exceptionally high numbers were found in some instances even after three to four and one-half months' storage, indicating that gross contamination must have existed in some of these samples prior to freezing. Factors responsible for this contamination have been presented in previous papers by Gunderson, Rose, and

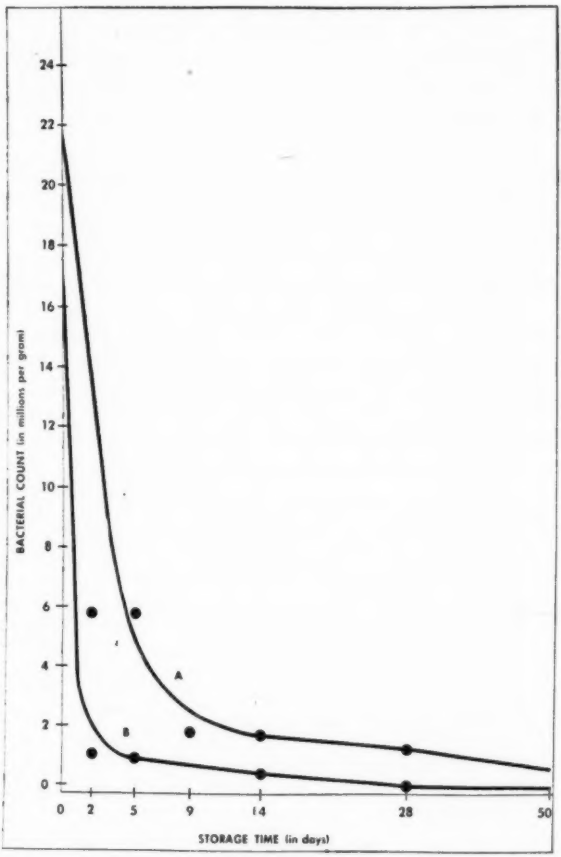

FIG. 3. The survival of the two enteric pathogens in chicken chow mein containing the natural flora of this material and stored at —14°F. A = *Salmonella paratyphi A*. B = *Salmonella newport*.

Henn (1947) and Rose and Gunderson (1947). The coliform counts, of course, are unreasonably high, especially in view of the recently proposed standard of a M.P.N. of 100 per 100 grams of frozen food, Fitzgerald (1947).

The hemolytic *Staphylococcus* counts obtained are only rough estimates because of the heavy growth of other microorganisms, but they

were found to vary from zero to 20,000 per gram. Most of the samples contained no hemolytic *Staphylococcus*. Hemolytic *Staphylococcus aureus* giving a positive reaction on Stone's extract gelatin agar were isolated from seven samples. It is particularly interesting that all of the samples of chicken salad contained hemolytic *Staphylococcus*, five of them containing hemolytic *Staphylococcus aureus*. Chicken salad is not cooked prior to eating and may rest for some time in the customer's refrigerator prior to use. The important question of whether 20 to 16,000 hemolytic *Staphylococcus aureus* are of significance in the mixed flora of this material cannot be answered at this time, but past experiences with salad-borne *Staphylococcus* food poisoning dictates the need for prevention of this sort of contamination.

DISCUSSION

Pathogenic enteric bacilli are killed rapidly in chicken chow mein stored at —14°F. during the first five days. After that, the rate of death drops off until a more or less resistant population remains. The normal saprophytic flora appears to be killed less rapidly. In either instance the numbers remaining after any given storage time depend primarily upon the initial contamination. When this is high even storage for as long as nine months does not assure a low count. A combination of the economic need for rapid turnover and spoilage owing to autocatalytic fat decomposition prevents holding chicken-containing foods longer than nine months to one year. The producer cannot rely on prolonged storage to reduce bacterial counts and, thus, is forced to adopt sanitary production methods if he desires to market a bacteriologically acceptable product.

The data presented do not furnish incontrovertible evidence that emulsified fat and other colloidal material contained in chicken chow mein protect bacteria from cold. However, when the 0.2, 0.03, 0.04, and 10 per cent seven-day survival of *Escherichia coli*, *Salmonella typhimurium*, *Salmonella paratyphi B*, and *Eberthella typhi* in cherry juice, reported by Wallace and Park (1933), is compared with the 6, 71, 40, and 13 per cent nine-day survival of the same organisms in chicken chow mein reported here, colloidal protection becomes a distinct possibility. In addition, Yesair, Bohrer, and Cameron (1946) have reported that fats protect *Micrococcus* strains from the lethal action of heat. It is possible that a similar protection from cold may be demonstrable. There is need for a rather thorough study into the effect of physical factors on bacterial survival in fresh-frozen foods.

SUMMARY

Death rate of six strains of *Salmonella* stored in sterile chicken chow mein at —25.5°C.(—14°F.) is essentially proportional to the number of viable bacteria present during the early stages of storage, after which the death rate is less rapid. Twenty per cent of the initial inoculum of *Salmonella typhimurium* survived nine months' storage.

The ability of *Escherichia coli* and *Aerobacter aerogenes* to grow on violet red bile agar decreases progressively with storage in chicken chow mein until only 12 to 25 per cent of the viable cells present are able to initiate growth.

In some instances, exceptionally high numbers of bacteria were found in commercially produced chicken chow mein and chicken salad despite storage at —12 to —14°F. for periods up to five and one-half months. Five of eight samples of chicken salad contained potential food-poisoning hemolytic *Staphylococcus aureus*.

Cold storage cannot "pasteurize" frozen food.

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HYDROXYMETHYLFURFURAL IN CALIFORNIA WINES

MAYNARD A. AMERINE
University of California, Davis, California

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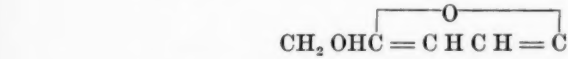
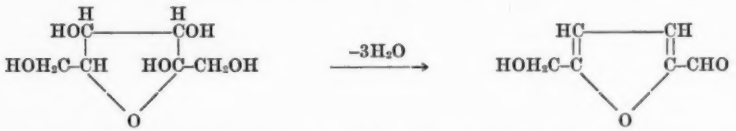
Reference was made briefly in a previous publication by Joslyn and Amerine (1941) to the presence and determination of hydroxymethylfurfural in California wines. This substance is formed by heating keto-hexose sugars in acid solution. The commonly accepted formula is



The formation from levulose as a dehydration process is more easily understood when the sugar is written with a furanose ring structure:



Hmf (as it will be abbreviated in this article) is soluble in water and has a neutral reaction. It is also soluble in ethyl and methyl alcohols, acetone, ether, chloroform, and benzene, but only slightly so in carbon tetrachloride or petrol ether. Middendorp (1919) found that pure crystalline hmf melted at 31.5°C.(88.2°F.).

In grapes or sweet wine hmf could originate from the levulose present since about 50 per cent of the sugar in grapes is levulose. Neither grapes nor wine are very acid, pH 3 to 4, but they apparently contain sufficient acid to favor the formation of hmf under appropriate temperature conditions. The necessary conditions which might give rise to its presence in wines are use of dried grapes; addition of caramel, of reduced or boiled-down musts, or of grape concentrate; heating of the musts or wines; or very long aging under warm conditions.

Since natural wines should not be subjected to these conditions the presence of hmf in Portuguese ports has been taken by Kruisheer, Vorstman, and Kniphorst (1935) and by Botelho (1938) as evidence of blending with reduced musts or with caramel. Very old dessert wines did not, however, show increased hmf as did old honeys. The amounts found in genuine port wines were small, 0 to 24 mgm. per liter. A port to which a small amount of concentrate admittedly had been added contained 46 mgm. per liter. The Dutch workers (Kruisheer *et al.*) mentioned that a few of the commercial ports imported into Holland contained more hmf than genuine ports, especially the white ports. Addition of reduced musts, concentrate, or caramel to port is prohibited by Portuguese law, according to Serra and Rodrigues (1938), so that such concentrations of hmf apparently constituted sophistication. In Tarragona and Malaga wines, to which reduced must *(arrope)* is generally added,

much larger amounts were found, 94 to 1,000 mgm. per liter. Botelho (1938) found no hmf in 27 authentic ports of the years 1933, 1928, 1913, and 1888, but wines to which one per cent of reduced must or caramel were added contained 110 and 66 mgm. per liter.

Several methods for qualitative detection of hmf have been suggested. Middendorp (1919) gives the melting points of several derivatives of furfural and hmf, including those with phenylhydrazine, p-nitrophenylhydrazine, oxime, and semicarbazone, and states that Fiehe's reaction is specific for hmf. This reaction depends on the development of a red color in the presence of resorcinol in concentrated hydrochloric acid. A less-specific qualitative procedure is the phloroglucinol reaction which gives a red to black precipitate with hmf in a strongly acid solution.

Three quantitative procedures have been proposed. Von Fellenberg (1934) used phloroglucinol to precipitate the hmf. The amount of purified precipitate was determined by chromic acid oxidation and measurement of the residual chromic acid. Kruisheer and co-workers (1935) criticize this procedure as giving high results. They extracted the wine with ether at pH 7. The ether extract was treated with sodium sulfate and petrol ether and dried on a water bath in oxygen-free carbon dioxide. The residue was dissolved in hydrochloric acid, filtered, and phloroglucinol was added. The precipitate was filtered, dried at 100°C.(212°F.), and the weight of hmf determined from a table which was established by using pure hmf. Maaskant (1936) suggested p-nitrophenylhydrazine for the quantitative determination of hmf. It may also be used in the qualitative detection of hmf.

EXPERIMENTAL PROCEDURE

The tests outlined here were made for the purpose of detecting whether hydroxymethylfurfural is present in commercial California wines and, if so, in what types. As a check, experimental wines of known history were used for comparison. The procedures used were Fiehe's reaction, a qualitative phloroglucinol procedure, and some tests with p-nitrophenylhydrazine.

Fiehe's reaction was carried out as follows: 10 ml. of wine were extracted twice with 10 ml. of anhydrous ether in separatory funnels. The ether extracts were placed in a porcelain evaporating dish and the ether allowed to evaporate at room temperature. Five ml. of 30 per cent hydrochloric acid were then added, followed by two ml. of freshly prepared Fiehe's solution (one gm. of resorcinol in 100 ml. of concentrated hydrochloric acid). If hmf is present the contents of the dish become red. The amount and depth of color is a measure of the quantity of hmf present. The color was estimated as 0, +, ++, or +++ for none, slight, moderate, or large amounts.

The phloroglucinol reaction was made directly in the wine. White wines were used without previous treatment but red wines were first decolorized with bone charcoal. (Vegetable chars were found to give low results.) Two ml. of the white or decolorized wine was placed in a test tube, one ml. of freshly prepared phloroglucinol solution (one gm. in 100 ml. of 30 per cent hydrochloric acid) was added, followed by two ml. of the same strength acid. A few minutes after shaking a red or deep orange color developed in the wines containing hmf. Decolorized wines

required up to an hour for the development of the color. The amount of color precipitated gave a rough measure of the hmf present. The color was estimated as 0, +, ++, or +++ for none, slight, moderate, or large amounts.

The p-nitrophenylhydrazine procedure was conducted as follows: The wine was extracted twice with 10 ml. of anhydrous ether. The ether extracts were evaporated nearly to dryness under nitrogen and 10 ml. of water was added. About 0.5 gm. of p-nitrophenylhydrazine was dissolved in 50 ml. of 30 per cent acetic acid by boiling. The solution was filtered and 10 ml. of the filtered solution added to the water solution of the extract. A reddish coloration and precipitate developed in the samples containing hmf.

Some quantitative tests were also performed using the extraction apparatus previously described by Amerine (1944) and the Kruisheer *et al.* (1935) procedure. Twenty-five ml. of wine were neutralized to a pH of 7 with 0.1 *N* sodium hydroxide using a Beckman pH meter. The sample was placed in the extraction apparatus and extracted six hours with ether at the rate of about 300 ml. per hour. The ether was poured off the wine, 25 ml. of petrol ether and 10 grams of anhydrous sodium sulfate were added to the ether, and the mixture was allowed to set 24 hours. It was filtered twice through a sintered glass filter, the filter being washed each time with a 1:1 ether-petrol ether mixture. The filtrate was evaporated nearly to dryness at room temperature under a stream of nitrogen and then rapidly to dryness under nitrogen on a boiling water bath. The residue was taken up in 10 ml. of 15 per cent hydrochloric acid and filtered. The residue was rewashed with 10 ml. of acid and filtered and 0.25 gm. of phloroglucinol dissolved in 25 ml. of 15 per cent hydrochloric acid was added. After two hours the precipitate was filtered onto a weighed, sintered-glass filter, dried at 100° C. and reweighed. The weight of the phloroglucinol-hmf precipitate was converted to milligrams of hmf by reference to Kruisheer's table.

Since levulinic acid is a further product of hexose dehydration, tests as to its possible interference in the resorcinol or phloroglucinol procedures were made. No reaction with dilute levulinic acid solutions was obtained. No reaction was obtained by either procedure using solutions of grape-seed tannin. Diluted caramel solutions gave a very large reaction, however.

The preparation of the hmf-p-nitrophenylhydrazone was conducted as follows: A stock hmf solution was prepared by extracting a sweet wine which had been heated for three months at 54.4°C.(130°F.) with ether. An aliquot of the extract was diluted with water and the ether evaporated off under a stream of nitrogen. To the water solution 10 ml. of a solution of p-nitrophenylhydrazine was then added. (This solution was prepared by dissolving 0.70 gm. in 50 ml. of 30 per cent acetic acid, heating to boiling, and filtering twice.) There was an immediate clouding and within an hour, a precipitation. After 24 hours the solution was filtered through a sintered glass filter, washed with water, and dried at 105°C.(221°F.). The precipitate was twice recrystallized by dissolving in hot acetic acid and adding water. The melting point was determined several times and was 175 to 180°C.(347 to 356°F.). This is somewhat below the commonly

accepted value of 183 to 184°C. (361.4 to 363.2°F.). Possibly there are impurities present which account for the reduction in melting point of the hydrazone.

The wines used for the original tests were the commercial dessert wines submitted for judging at the 1939 Golden Gate International Exposition and a few submitted for class use by various California producers in 1946. The experimental wines used for comparison were all produced from grapes grown in the University vineyard at Davis and vinified and stored under known conditions. No concentrate, reduced must, or caramel was used in treating any of the University wines; the results are given (Tables 1 and 2).

TABLE 1

Hydroxymethylfurfural Content of Commercial California Wines

Type	Source	Number of samples	Fiehe's test				Phloroglucinol test			
			Reaction of samples				Reaction of samples			
			0	+	++	+++	0	+	++	+++
Dry sherry	GGIE	19	0	2	13	4	0	7	10	2
Dry sherry	Class [1]	4	1	1	2	0	....	....	....	....
Sherry	GGIE	29	0	3	17	9	0	6	19	4
Sherry	Class	10	0	3	4	3	....	....	....	....
Tokay	GGIE	6	3	2	1	0	....	....	....	....
Madeira	GGIE	1	0	0	1	0	....	....	....	....
Muscatel	GGIE	16	5	7	3	1	10	5	1	0
Muscatel	Class [1]	7	1	3	3	0	....	....	....	....
Angelica	GGIE	15	10	3	2	0	11	1	2	1
Angelica	Class [1]	3	0	2	1	0	....	....	....	....
White port	GGIE	6	6	0	0	0	....	....	....	....
Malaga	GGIE	3	1	1	1	0	....	....	....	....
Marsala	GGIE	2	1	0	1	0	....	....	....	....
Port	GGIE	25	19	4	2	0	20	5	0	0
Port	Class [1]	8	3	5	0	0	....	....	....	....
Table wines	Class [1]	43	35	8	0	0	....	....	....	....

[1] Commercial samples from industry for class purposes.

TABLE 2

Hydroxymethylfurfural Content of Wines of Known Origin and Treatment

Variety	Original Balling	Treatment	Sugar content of wine	Fiehe's reaction	Phloroglucinol reaction
			gm./100 ml.		
Mission	25.4	None	0.35	0	0
Mission	25.5	4 months at 110°F.	0.35	Trace	0
Criolla de Vino	23.5	None	0.91	(?)	0
Criolla de Vino	23.5	4 months at 110°F.	0.91	+	0
Inzolia	23.7	None	2.20	0	0
Inzolia	23.7	3 months at 110°F.[1]	2.20	+	++
Criolla de Mesa	25.8	None	4.10	Trace	0
Criolla de Mesa	25.8	4 months at 110°F.	4.10	++	+
Verdelho	28.5	None	7.60	0	0
Verdelho	28.5	4 months at 110°F.	7.60	+++	++
Grillo	28.5	None	0.51[2]	0	0
Grillo	28.5	4 months at 110°F.	12.20	+++	++

[1] Aerated three times during treatment. [2] No strictly comparable untreated sample available.

DISCUSSION

All except one of the 62 samples of commercial California sherry and dry sherry examined contained hmf, some in considerable amounts. This is to be expected with the present general use of baking for producing California-type sherry. Too few samples of the so-called California Tokay, Madeira, Malaga, and Marsala were examined to make any general statement. A sufficient number gave a positive reaction to indicate, in general, either heating, use of concentrate, or blending with California sherry in producing these types. The California port and angelica wines, however, frequently contain none or only small amounts of hmf. It is of interest to note that California white port is free of hmf, indicating no heating or the removal of any hmf originally present by the activated vegetable charcoals commonly used in preparing this type of wine. The table wines were largely free of hmf and the samples giving a positive test contained only very small amounts. The presence of hmf in wines of low sugar content was unexpected, but some of the samples contained sufficient sugar to account for its production if the wines had been heated. The use of heating procedures to age quickly wines other than sherry is becoming more general even though the quality of some wines may be adversely affected.

TABLE 3

Quantitative Determination of Hydroxymethylfurfural

Wine	Amount	Hours extracted	Rate per hour	Precipitate	Hmf. per liter
	ml.		*ml.*	*mg.*	*mg.*
Old sherry	20	4	300	10.9	305
Sherry No. 1	50	9	250	7.4	98
Sherry No. 2	25	6	300	2.0	92
Sherry No. 3	25	6	300	0.8	32(?)
Sherry No. 4	25	8	300	4.9	152
Sherry No. 5	25	20	300	12.9	284

The effect of heating in the formation of hmf is clearly shown (Table 2). In each case, except one, the original wine was divided into two lots, one of which was held at 12.8°C.(55°F.), while the other was placed in glass in a hot room held at 43.3 to 48.9°C.(110 to 120°F.). All of the heated samples gave some sort of positive reaction with Fiehe's solution, but the reaction was much less when wines of low sugar content were heated, and negative results were obtained when the phloroglucinol test was applied to the low sugar samples. The high sugar-treated samples gave the most significant tests. The untreated samples were all negative except for a trace in one and the questionable occurrence in another. This series of tests shows clearly that it is the heating of wines containing sugar which leads to the formation of ether-extractable substances which react with Fiehe's solution or phloroglucinol—presumably hmf. The quantitative results indicate something of the range in amounts of hmf—32 to 305 mgm. per liter—to be expected in baked wines using the phloroglucinol procedure.

SUMMARY

The presence of levulose dehydration products, probably largely hydroxymethylfurfural, in baked wines is confirmed for California wines.

Qualitative and quantitative tests are described. The general occurrence of this substance in California dessert wines indicates the widespread use of heat treatments in the early finishing of these types.

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EFFECT OF RATE OF FREEZING ON BACTERIAL CONTENT OF FROZEN VEGETABLES[1]

WM. P. VAN ESELTINE, LOIS F. NELLIS, F. A. LEE, AND G. J. HUCKER

Division of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York

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While there have been numerous reports regarding the effect of freezing on pathogenic and food-spoilage organisms associated with frozen foods, little attention has been paid to the effect of rate of freezing on microbial content of such products. This factor becomes increasingly interesting with the advent of numerous farm and home freezing units and in view of the recent report by Lee, Gortner, and Whitcombe (1946) that peas and snap beans may show few differences in vitamin content or palatability whether frozen very slowly, very rapidly, or at intermediate rates.

Smart and Brunstetter (1936, 1937) have shown that scalding and blanching reduced the microbial content of lima beans, spinach, and kale more than 90 per cent, and that subsequent freezing and storage further reduced the number of organisms more than 99 per cent. No mention is made, however, of the effect of rate of freezing on microbial flora, and further investigation of this point seemed advisable.

EXPERIMENTAL PROCEDURE

Two products were selected for this investigation: peas, representing a hard, uncut product relatively free from juices; and sweet corn, representing a soft, cut product with an abundance of juices affording a better medium for bacterial growth. Peas of the Perfection variety were obtained from a nearby viner station and brought immediately to the laboratory where they were washed, blanched in rapidly boiling water for one minute, cooled in cold water, drained, mixed thoroughly, and packaged in pint Vapo-Pak containers. Corn of the Golden Cross variety was secured from a nearby grower and was brought immediately to the laboratory where it was husked, blanched in rapidly boiling water for seven and one-half minutes, cooled for an equal period of seven and one-half minutes in cold sprays and in cold water, cut from the cob, thoroughly mixed, and likewise packaged in pint Vapo-Pak containers.

Method of Freezing: A Brown recording potentiometer equipped with thermocouples of iron and constantan was used; its range is —101.1 to 148.9°C.(—150 to 300°F.). The chart speed can be altered, and was set at two inches an hour. The thermocouple leads were covered with Irvolite extruded, plastic tubing, type XTE 30, black, size 14. A home freezing

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cabinet was provided that maintained a temperature of —17.8°C.(0°F.). In addition, a Sub Zero test cabinet made by the American Instrument Company was used, which yielded a temperature of —51.1°C.(—60°F.). An 18-inch fan was used in the Sub Zero cabinet and was supported so that the packages would receive an air blast from above which provided a uniform air velocity for all boxes. Preliminary runs showed that the contents of these boxes froze in substantially the same time. Plastic-coated, pint-sized Vapocans were used for this work. Fastest freezing was obtained by immersing the loose, blanched vegetable in liquid air, followed by packaging in moisture-vapor-proof cartons and storage at 0°F. for the corn, and —23.3°C.(—10°F.) for the peas. In the other methods of freezing, the blanched vegetable was packaged in the usual fashion before freezing. One set was frozen in the Sub Zero cabinet where freezing was hastened by the air blast. Another set was frozen in still air at 0°F. A third set was left in the same room, but the cartons were put into corrugated, insulated boxes. Eight cartons were placed in the insulated box, in which one-half inch of wool lining was used, and the eight pint boxes were fitted snugly inside. One box of the upper four was fitted with a thermocouple at the lower inner corner and another at the center of the outside surface. Experiment showed that such placement measured accurately the extremes of temperature change in the entire cube of eight boxes.

TABLE 1

Effect of Method of Plating and Composition of Media on Bacteria Counts Obtained From Commercially Frozen Peas

Samples of commercially frozen peas	Rinse count (per gram) (usual method)		Disintegrated count (per gram) (revised procedure)	
	Nutrient agar	TGYE agar	Nutrient agar	TGYE agar
Brand "A"				
1	6,700 [1]	4,600 [1]	9,500 [1]	9,900 [1]
2	3,650	2,970	10,300	8,400
3	1,455	1,535	5,600	4,900
Brand "B"				
1	13,050	11,850	42,000	48,000
2	14,250	13,950	45,500	65,500
3	5,150	4,450	24,500	26,700
Brand "C"				
1	235,000	295,000	435,000	2,550,000?
2	99,000	223,500	410,000	785,000
3	182,000	293,000	416,000	620,000

[1] Each count represents the mean between duplicate plates.

The thermocouples were held in place by using wooden clamps grasping the insulated lead-in wires. With this device little difficulty was encountered in use. The boxes fitted with thermocouples were not sampled for bacteriological analysis, and when the freezing was finished, they were discarded.

Bacteriological Technique: In the usual procedure for the bacteriological examination of frozen foods, outlined by Sanderson (1941), 100 grams of the sample are placed in 100 ml. of sterile water and are shaken

for one minute. From these washings a 1:1,000 dilution is prepared and one ml. of this final dilution is placed in each of two petri dishes. Fifteen ml. of nutrient agar are added and the plates are incubated at 25°C. (77°F.) for 72 hours. A count of less than 100,000 organisms per gram is usually considered satisfactory.

We have found, however, that samples of three different brands of commercially frozen peas yielded higher bacteria counts when the peas in sterile water were disintegrated in a sterile Waring blendor and the platings were made from the resulting suspension. These higher counts were presumably the result of better freeing of bacteria from the peas and the breaking up of clumps of organisms. The results of these experiments are summarized (Table 1); it is evident that the use of tryptone-glucose-yeast extract (TGYE) agar gave higher counts in most instances where the peas were disintegrated. Accordingly, the following procedure was used in these experiments for plating the peas and corn:

Twenty grams of the product were placed in a sterile Waring blendor with 200 ml. of sterile tap water and disintegrated for five minutes. At the end of this time, dilutions of the suspension of 1:10, 1:100, 1:1,000, and 1:10,000 were plated, using a medium composed of tryptone, 5 gm.; glucose, 1 gm.; yeast extract, 2.5 gm.; agar, 15 gm.; and distilled water 1,000 ml. All plates were incubated at 25°C. for 72 hours before counting. Dilutions of 1:10, 1:100, and 1:1,000 were also plated, using wort agar (Difco) to obtain counts of yeasts and molds.

Samples of each product were taken for bacteriological analysis at the start of the experiment, after freezing in liquid air, and at various intervals during freezing at the slower rates. Duplicate samples of peas and triplicate samples of corn were taken for each analysis.

RESULTS AND DISCUSSION

Bacteria, yeast, and mold counts obtained for the peas and corn when ready for freezing, at intervals during freezing, and again at the end of freezing, are given (Tables 2 and 3). It is apparent that freezing very rapidly in liquid air does not materially change the bacterial content of the product. Luyet and Gehenio (1940) stated that during such rapid freezing the water in the cell is not changed to ice crystals but to a glass-like amorphous mass (vitreous state), and this results in less injury than freezing at slower rates. In the present studies the bacterial count of the peas remained the same while that of the corn dropped slightly. Although this method of freezing is probably not a practical one, it could be expected to yield a product having nearly the same bacterial content as before freezing.

Injury to the cells by ice crystals during the process of slower freezing would result in lower bacterial counts; however, in these methods the bacteria have an opportunity to grow before the product reaches the freezing temperature. This possible growth period is of prime importance in the case of farm and home freezers where the rate of freezing is relatively slow.

Frozen at —60°F. in air blast, the peas showed a slight increase in bacterial content, while the corn showed a slight decrease. When temperature and bacterial count were plotted together on the same graph (Figs. 1 and 4), there appeared to be no direct correlation. It is known, however,

that freezing will stop the growth of the organisms and kill some of them, and it is apparent from these graphs that the foods reached the freezing temperature in a half hour or an hour. Not much multiplication can be expected in this time since the temperature is constantly decreasing. Consequently, one can expect the procedure to result in a bacterial content only slightly different from the initial. If multiplication is fairly rapid, the count may be slightly higher; but if multiplication is slow and freezing kills a large number of organisms, the count may be slightly lower.

TABLE 2

Bacteria, Yeast, and Mold Counts on Peas Frozen at Different Rates

Samples	Temperature (°F.) at time sample was taken		Bacteria count per gram	Yeast count per gram	Mold count per gram
	Outside of box	Center of box			
Control (at start of freezing)	72	72	5,800 [1]	350 [1]	15 [1]
Samples Frozen in Liquid Air					
Immediately after freezing	.....	.....	5,800	335	20
After 5 months' storage at —10°F. ambient temp.	.....	.....	970	20	<10
Samples Frozen in Air Blast at —51.1°C. (—60°F.)					
After freezing for 44 minutes	Below 0	29.5	7,500	430	10
2 hours (at end of freezing)	Below 0	Below 0	10,800	185	20
After 5 months' storage at —10°F. ambient temp.	.....	.....	4,617	57	<10
Samples Frozen in Still Air at —17.8°C. (0°F.)					
After freezing for 1 hour	31.00	40.00	10,100	215	15
2 hours	28.00	31.50	10,500	165	<10
3 hours	24.00	31.50	13,500	230	20
4 hours	21.00	31.25	17,200	255	20
7 hours, 54 minutes	2.50	4.00	11,450	805	<10
11 hours, 49 minutes (at end of freezing)	—2.25	—2.00	11,700	240	<10
After 5 months' storage at —10°F. ambient temp.	.....	.....	775	17	<10
Samples Frozen in Insulated Boxes in Still Air at 0°F.					
After freezing for 4 hours	32.25	42.00	13,200	295	20
7 hours, 54 minutes	29.25	31.00	15,150	500	<10
13 hours, 44 minutes	24.00	30.75	47,000	76,100	30
20 hours, 44 minutes	19.75	28.75	20,100	10,800	25
25 hours, 44 minutes	14.75	21.00	48,250	57,400	35
38 hours, 29 minutes (at end of freezing)	—2.25	—2.25	12,600	340	30
After 5 months' storage at —10°F. ambient temp.	.....	.....	737	15	<10

[1] Each count represents the mean between duplicate samples plated.

When peas and corn were frozen at 0°F. in still air, a few hours were required for the material to reach freezing temperature (32°F.) through-

out. Consequently, multiplication of microorganisms was possible for a short time after the foods were placed in the freezer. Growth ceased as the temperature reached freezing (Figs. 2 and 5); then the count decreased somewhat as freezing killed some of the cells. It is surprising that the peas, which apparently represented a poorer growth medium, showed a greater increase in bacterial content than the corn and had a higher final than initial count. In the case of the corn there was less multiplication, and the number of cells killed by freezing was sufficient to reduce the final count to a level lower than the initial count.

TABLE 3

Bacteria, Yeast, and Mold Counts on Corn Frozen at Different Rates

Samples	Temperature (°F.) at time sample was taken		Bacteria count per gram	Yeast count per gram	Mold count per gram
	Outside of box	Center of box			
Control (at start of freezing)	71	71	135,000 [1]	6,100 [1]	155 [1]
Samples Frozen in Liquid Air					
Immediately after freezing	.....	.....	101,667	1,567	87
After 7 months' storage at 0°F. ambient temp.	.....	.....	104,500	70	160
Samples Frozen in Air Blast at —51.1°C. (—60°F.)					
After freezing for 1 hour	26	33	127,667	6,233	213
3 hours, (at end of freezing)	—18	—18	127,000	6,450	173
After 7 months' storage at 0°F. ambient temp.	.....	.....	107,000	2,000	110
Samples Frozen in Still Air at —17.8°C. (0°F.)					
After freezing for 1 hour	20.00	41.00	139,333	7,333	150
3 hours	19.75	31.50	114,667	8,133	227
8 hours	10.00	14.25	115,000	8,600	167
15 hours (at end of freezing)	—2.00	—2.25	95,333	5,767	207
After 7 months' storage at 0°F. ambient temp.	.....	.....	76,667	3,400	167
Samples Frozen in Insulated Boxes in Still Air at 0°F.					
After freezing for 5 hours, 30 minutes	36.25	38.00	128,667	10,833	187
15 hours	29.50	31.50	155,667	10,250	253
25 hours	26.25	29.00	119,000	8,467	173
40 hours	9.00	10.00	108,333	8,000	163
47 hours, 14 minutes	2.00	3.25	109,667	8,867	133
50 hours (at end of freezing)	—0.25	—0.25	125,333	12,500	150
After 7 months' storage at 0°F. ambient temp.	.....	.....	105,000	6,200	150

[1] Each count represents the average of triplicate samples plated.

Freezing at 0°F. with the samples packed in insulated boxes resulted in a very slow rate of freezing. During the relatively long time which elapsed before the material reached the freezing temperature, considerable multiplication of the organisms occurred (Figs. 3 and 6). After 15 to 25 hours

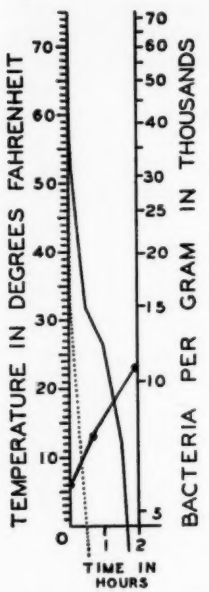

FIG. 1. Temperature and bacteria count of peas frozen at −51.1° C.(−60°F.) in an air blast.

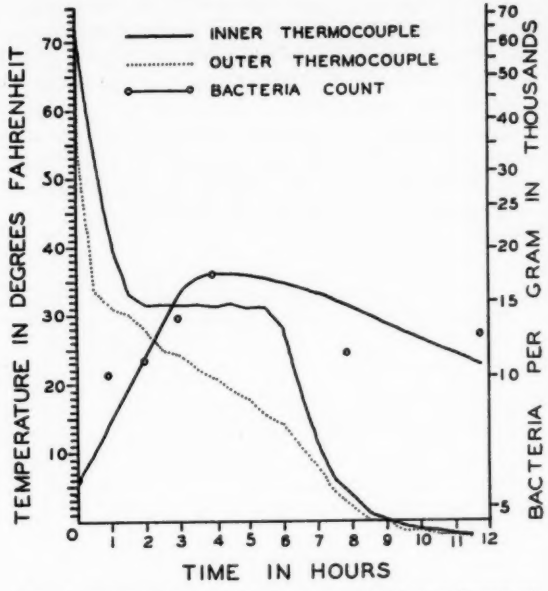

FIG. 2. Temperature and bacteria count of peas frozen at −17.8°C.(0°F.) in still air.

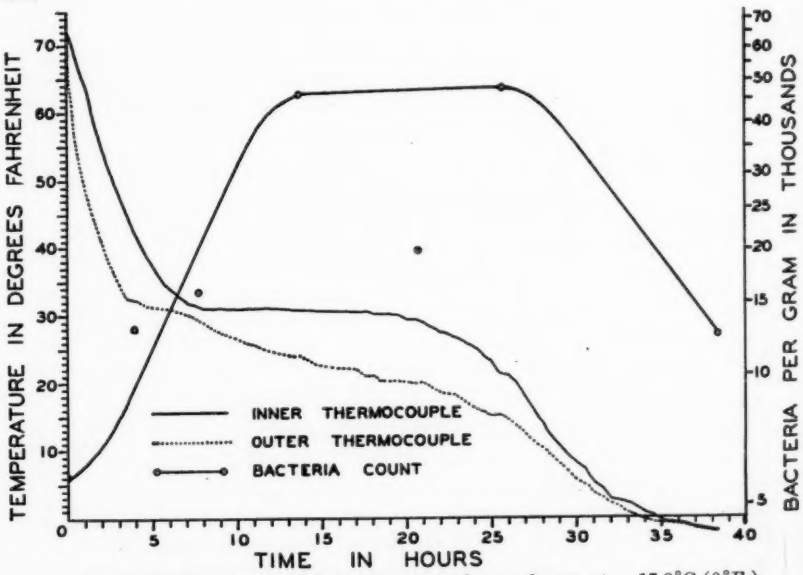

FIG. 3. Temperature and bacteria count of peas frozen at −17.8°C.(0°F.) in insulated boxes.

the temperature reached 32°F., growth ceased, and the ordeal of freezing again reduced the bacterial content. Since the foods were held in the freezing chamber until the center of the packages reached 0°F., it is possible that the slow death of frozen bacteria, [Rahn (1945)], participates in the reduction of the bacterial content towards the end of the experiment. In this case again, the final count on the peas was slightly higher than the initial count, and the final count on the corn was lower than the initial.

Also given (Tables 2 and 3) are the counts obtained on each product, frozen at the different rates, after the frozen samples had been stored for approximately six months. These indicate, as was expected, that there is a slow death of the frozen organisms during storage. The work of Rahn and Bigwood (1939) suggests that this slow death may be due to an oxidative process. The death rate in the present studies appeared to approximate the order of 90 per cent in about six months for the peas, but in the case of the corn the rate was much slower, presumably because the bacteria were protected by the medium of corn juices in which they were suspended.

TABLE 4

Bacterial Content of Three Samples From Same Brand of Commercially Frozen Corn Obtained on Open Market at Same Time

Sample	Bacteria count per gram
1	4,400,000 [1]
2	960,000
3	7,900,000

[1] Each count represents the mean between duplicate plate counts.

In a general way the yeast and mold counts tended to follow the bacterial count for each of the methods of freezing, but the mold count, especially in the case of the peas, appeared too low to be significant.

It was noted that in these experiments the final counts, after freezing at the several different rates, were not widely different, indicating that the rate of freezing in farm or home freezers may be sufficient for the production of frozen foods of good quality. A comparison of the bacterial counts obtained upon commercial peas (Table 1) with the counts obtained on peas frozen in the laboratory (Table 2) indicates that home-frozen products may have a lower bacterial count than the commercially packaged product, provided the food is taken from the garden and placed in the freezer as quickly as possible. The same effect is apparent with corn. Corn frozen in the laboratory (Table 3) had a lower bacterial count than three samples of commercially frozen corn (Table 4). This is undoubtedly due to the fact that the corn frozen in the laboratory could be taken from the cob and placed in a freezer in a limited amount of time, whereas in large-scale commercial production some time may pass before the packaged corn enters the freezers. Commercially frozen corn is likely to pick up contamination from the cutters. Also, home-frozen products can be maintained in the frozen condition, but it is likely that at least some commercially frozen foods become partly thawed some time previous to the time of purchase.

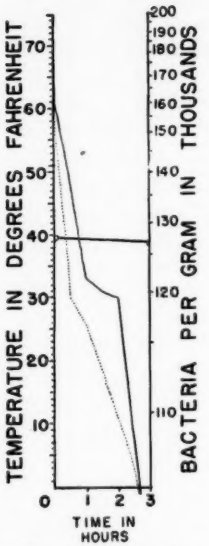

FIG. 4. Temperature and bacteria count of corn frozen at —51.1° C.(—60°F.) in an air blast.

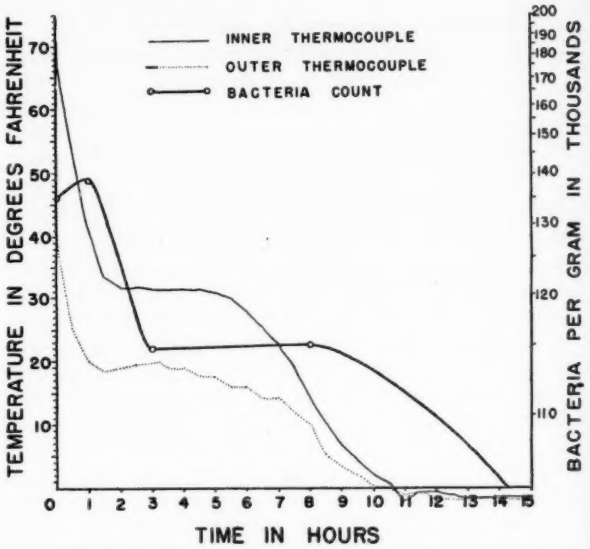

FIG. 5. Temperature and bacteria count of corn frozen at —17.8°C.(0°F.) in still air.

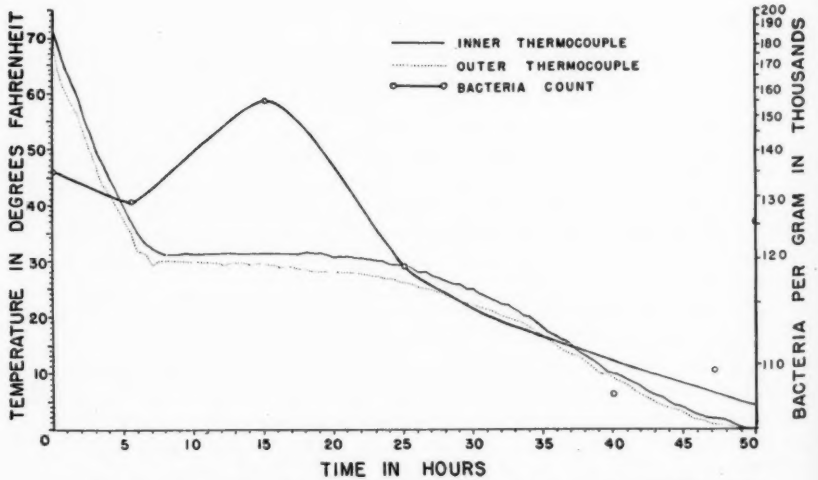

FIG. 6. Temperature and bacteria count of corn frozen at —17.8°C.(0°F.) in insulated boxes.

The placing of the blanched food in the freezer as quickly as possible is essential. The enormous number of bacteria that can develop on blanched peas left standing at the temperature of a hot kitchen in summer is indicated (Table 5).

It must be emphasized that in addition to placing foods in the freezer quickly, the freezer must not be packed too full. If cartons are jammed into a freezer until it is packed solid, the packages at the center of the stack may not reach freezing temperature for a considerable length of time. This is approximated by the condition developed in our experiments where the foods were frozen after being packed in insulated boxes. Growth of bacteria occurred for nearly 15 hours (Figs. 3 and 6) and although many of these were apparently killed by freezing, it is safer not to depend upon this effect, and the condition is to be avoided.

TABLE 5

Growth of Bacteria, Yeasts, and Molds on Packaged Peas Held at 32.2°C.(90°F.)

Samples	Bacteria count per gram	Yeast count per gram	Mold count per gram
At start	5,800 [1]	350 [1]	15 [1]
After 3 hours, 44 minutes	106,000	17,850	10
After 7 hours, 15 minutes	34,750,000	4,980,000	Less than 100
After 11 hours, 54 minutes	1,635,000,000	.....	Less than 100
After 15 hours, 44 minutes	3,655,000,000	.....	100
After 20 hours, 9 minutes	3,160,000,000	.....	100
After 24 hours, 14 minutes	2,190,000,000	.....	400

[1] Each count represents the mean between duplicate samples.

SUMMARY AND CONCLUSIONS

Peas and whole-kernel corn were frozen very rapidly in liquid air, very slowly at 0°F. packed in insulated boxes, and at two intermediate rates.

Freezing in liquid air and in an air blast at —60°F. resulted in such quick freezing that the bacterial content of the foods was essentially unchanged.

Freezing at slower rates allowed multiplication of bacteria before the freezing temperature was reached, but of the increased numbers of bacteria, many were killed by the ordeal of freezing.

Freezing in still air at 0°F., a process which can be duplicated in farm or home freezers, seems practical from a bacteriological standpoint.

It is essential to package the blanched food and place it in the freezer as quickly as possible to prevent bacterial growth on the food.

It is important not to pack the freezer units tightly, as this will result in too-slow freezing of the packages in the center of the stack and bacterial multiplication can be expected to occur.

During storage there is a slow death of the bacteria in the frozen state.

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A NEW SPECIES OF TORULAE OCCURRING IN AND TRANSMITTED BY THE AMERICAN COCKROACH *PERIPLANETA AMERICANA* (LINN.)

WILLIAM L. OWEN
Consulting Bacteriologist,
Baton Rouge, Louisiana

AND

ROY L. MOBLEY
Assistant Bacteriologist,
Baton Rouge, Louisiana

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TORULA ACIDOPHILA (N. SP.)

In the course of maintaining the bacteriological control of a sirup product over a period of many years, the writers encountered a very unusual species of *Torula*, the original habitat of which was then unknown, and the determination of whose original source presented an elusive problem until somewhat recently. The morphological and physiological characteristics of *Torula acidophila* immediately suggested that it had not, heretofore, been described in the literature. Extended efforts to ascertain if other workers in this field had encountered it led to the belief that it had not previously been discovered and that it is definitely an addition to the species of yeasts that are now known.

The distinctive characteristics of *Torula acidophila* are (1) its diminutive size and (2) its tolerance of very high hydrogen-ion concentration. The cell dimensions of this species average 1.4 microns in diameter and 3.3 microns in length. Comparison with some of the largest species of bacteria, namely *Bacillus megatherium*, discloses that the two species are approximately of the same dimensions, the latter averaging from 1.0 to 1.5 microns in diameter and 3.0 to 3.6 microns in length. The following species, as reported in the literature, seem to be most closely related to *Torula acidophila* in size:

(1) *Torula Holmi* (Holm), Jorgensen 1909, isolated by Holm. Its dimensions are 3.5 microns, length of cells, 1.4 to 2.1 microns, width. This yeast, however, occurs only as oval or round cells, while *Torula acidophila* cells are chiefly elliptical.

(2) *Torula Kephir*, Heinze and Cohn (1908), isolated from Kephir. The dimensions of these cells are 3.5 microns, length and 2 to 3 microns, width. These cells are oval, however, rather than elliptical.

The distinctive physiological characteristic of *Torula acidophila* is its unusual tolerance of high concentrations of acid; the sirup in which it was first detected and in which it grows freely has a pH of 1 to 2. A review of the literature on the subject of this property of yeast reveals nothing comparable to the above-described species of *Torula*. As an example of the range of pH in which *Torula acidophila* is capable of inducing fermentation in sirups, the pH and titratable acidity of various sirups in which *Torula* species developed fermentation are shown (Table 1).

TABLE 1

Acidity of Sirups Undergoing Fermentation by Torula Species

Sample No.	pH	Titratable acidity (N/10 NaOH—10 c.c.)
1	2.60	22.0
2	1.90	18.0
3	1.80	20.0
4	1.80	26.0
5	1.90	22.0
6	1.60	14.0
7	1.65	12.0
8	2.10	12.0
9	1.50	14.0
10	1.60	21.0
11	2.00	15.5
12	1.70	16.0
13	1.65	19.0
14	1.50	19.0
15	1.50	15.0
16	1.50	13.0
17	1.60	20.0
18	1.50	18.5
19	1.65	17.0
20	1.80	15.0
Average	1.66	16.2

MORPHOLOGICAL CHARACTERISTICS

Vegetative cells, elliptical to cylindrical in shape, with ends rounded and varying in size, averaging 1.4 microns in diameter and 3.3 microns in length.

Ascopores not produced either on carrots, gypsum blocks, filter paper, gelatin, or potatoes.

Few granules to be observed; vacuoles very small and rarely observed. Glycogen absent at all stages of development.

CULTURAL CHARACTERISTICS

Agar colonies—punctiform to circular, entire color white, butyrous, opaque.

Giant colonies—15 mm. diameter, smooth, raised, lobate butyrous, unchanged, opaque.

Agar stroke—moderate, echinulate, dull, no chromogenesis, odor like that of mellow apples; consistency butyrous, medium unchanged.

Dextro nutrient gelatin stab—three days' room temperature, moderate growth, beaded; no liquefication; best growth in middle of media, media unchanged.

Dextrose broth—ring of colonies on tube at upper meniscus of medium; slight clouding; odor of crushed apples; flocculent sediment, abundant.

Agar colonies—circular, glistening, smooth edges.

Gelatin colonies—circular with smooth edges.

PHYSIOLOGICAL CHARACTERISTICS

Acetyl methyl-carbinol—negative five days; good growth glucose-peptone solution.

Indole—negative five days; good growth using tryptophane broth.

Produces acid from glucose, fructose, sucrose, maltose, mannose, arabinose.

Produces gas from glucose, fructose, mannose, sucrose, and maltose.

Does not produce acid or gas from rhamnose, l-xylose, galactose, lactose, raffinose, melezitose, starch, inulin, dextrin, glycogen, glycerol, erythritol, mannitol, sorbitol, dulcitol, inositol, or salicin.

Starch not hydrolyzed.

Litmus milk, flocculent growth, no curd, slightly acid in seven days.

Optimum pH 4.9.

Optimum temperature 32 to 34°C. (89.6 to 93.2°F.).

Habitat, acidified beverage sirup.

Van Lear and Morton (1923) investigated the influence of free acid upon saccharomycetes in 1922 and found the optimum pH for the various species of yeast studied to be 6.0 for yeast Frohberg and a distillery culture studied, in beer wort, and 4.6 for *Bretanomyces lambicus*.

Buromsky (1915) investigated the tolerance of yeast to various organic acids, including quinic, citric, malic, tartaric, and succinic, and found they were most easily adapted to the former and least capable of adapting themselves to tartaric acid. On the other hand, Zikes (1915) found that yeasts vary in sensitiveness to different organic acids, being very sensitive to butyric but more resistant to lactic and least sensitive to tartaric acid. They are very resistant, also, to malic, citric, and succinic and very sensitive to formic and oxalic acids.

Hagglund (1914) investigated the maximum hydrogen-ion concentration permitting yeast growth and found it to be at a pH of 2.36 to 3. Euler and Lindner (1915) and Euler and Emberg (1919) found that both top and bottom yeasts have a very wide range of optimum hydrogen-ion concentrations, varying from a pH of 3 to 7.5. Svanberg (1922) found the optimum for *Saccharomyces validus* and *Saccharomyces thermanitomum* to lie between pH 4 and 6, while for torulae, on the other hand, the range was from pH 2.5 to 6.

Even the final pH of yeast growth so far recorded in the literature does not reveal any instance of tolerance comparable to *Torula acidophila*; the lowest recorded by Svanberg is that of a top yeast in mash having an initial pH of 5.6 and a final pH of 2.87.

Mold fungi, which require as a rule higher hydrogen-ion concentrations for their development than either yeast or bacteria, have been thoroughly studied for their tolerance in this capacity by Johnson (1923), who found the minimum pH for the species studied to be 1.6. This represented the minima for *Aspergillus oryzae, Penicillium variable,* and *Aspergillus terricola.*

Clark and Lubs (1915) reported 1.7 as the final or terminal pH from mold growth for a seven-day growth of *Aspergillus niger* in a carbohydrate medium.

While the injurious effect of hydrogen-ion represents, to a large extent, the toxicity of an acid for microorganisms, it must not be overlooked that the undissociated molecule, or the anion, may in some instances be equally as or even more injurious than the hydrogen-ion.

That the composition of the substrate may tend to minimize or aggravate the pernicious effect of the hydrogen-ion concentration has been well established by numerous investigations. Rosenblatt and Rosenblatt (1910) have shown that a high concentration of sucrose increases the acid toler-

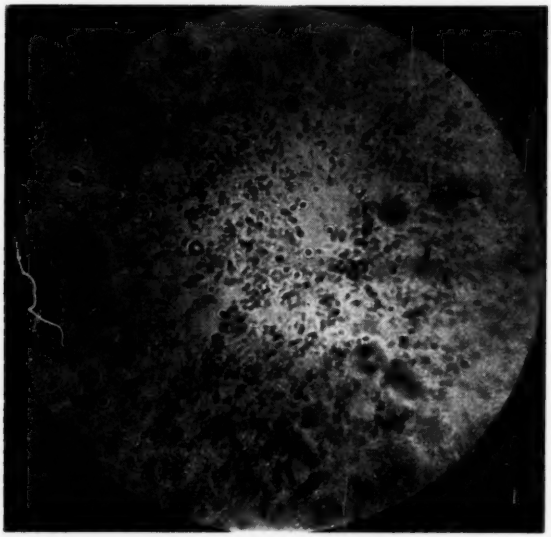

FIG. 1. *Torula acidophila* (N. sp.), isolated from sirup grown on dextrose agar (600 x).

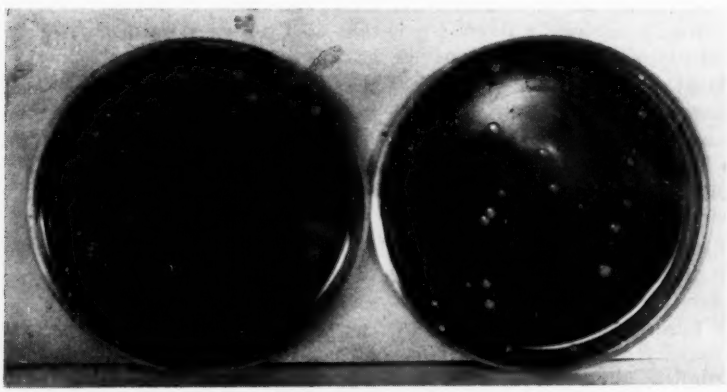

FIG. 2. Typical colonies on dextrose agar.

ance of yeast. Others have found different final pH's attained in media, varying only in the fermentable sugars present and irrespective of the fact that the sugars employed were equally fermentable by the organisms studied.

CHARACTERISTIC ALCOHOLIC FERMENTATION INDUCED BY *TORULA ACIDOPHILA*

The fermentation power of *Torula acidophila* is quite limited and the amount of alcohol produced from sugars is almost negative, as shown by the results of an incubation period of two weeks on a molasses mash inoculated with *Torula acidophila* (Table 2). The yeast grew well in this

TABLE 2

Results of a Two-Week Incubation Period of a Molasses Mash Inoculated With Torula acidophila

Sample No.	Inoculum (c.c.)	Loss in weight	Days	Titratable acidity		pH		Brix	
				Start	Finish	Start	Finish	Start	Finish
1	10	11.1	12	8.0	12.0	4.9	4.25	19.6	16.0
2	15	10.0	12	8.0	12.5	4.0	4.10	19.6	16.25
3	20	10.0	12	8.0	12.5	4.9	3.85	19.6	15.9
4	25	9.7	12	8.0	12.5	4.9	3.55	19.6	15.9

wort as indicated by the loss in weight from CO_2 evolution and from the increase in titratable acidity. The alcohol production was low, however, and not parallel to the weight loss; though the samples were distilled the alcohol was not determined, owing to the small amounts present.

HABITAT OF *TORULA ACIDOPHILA*

It might be supposed that highly resistant strains of torulae or wild strains of yeasts might be found in certain very highly acid foods, but again a search of the literature does not disclose any description of species comparable to *Torula acidophila* regarding their adaptitude to highly acid substrates. A possible source of such aciduric types of yeasts is sauerkraut, yet the Fred and Peterson (1922) investigation of this material, with reference to the occurrence of a pink yeast, showed the limits of hydrogen-ion concentration for this species to lie between 6.5 and 7.2 with a titratable acidity of 14.7 to 17.9.

The most likely source of *Torula acidophila* appears from our investigation to have been the following ingredients of the product: (1) refined cane sugar used in its manufacture, (2) essential oils, (3) caramel and coloring matter, (4) cotton bags in which the refined sugar was contained, (5) jute bags in which the raw sugar was brought to the refinery, and (6) dust in the plant.

A search of all these materials, covering a period of several years, failed to reveal the presence of this species of *Torula*, although other true and pseudo yeasts could usually be found in one or several of these materials. The jute bags in which raw sugar is shipped proved to be most interesting for the variety of microorganisms and especially the yeasts that they frequently contain and, in several instances, very small torulae. The latter, however, were much larger than the *Torula acidophila* cells

isolated from the sirup. The investigation of refined sugars used in the manufacture of this sirup did not reveal, in any instance, the presence of this specific organism, although many species of torulae were isolated from the hundreds of samples analyzed. Having exhausted all of the prospective sources of this contaminant through a thorough study of all materials utilized in the manufacture of the sirup used, in all the plants in which it was produced, the idea occurred to us that possibly this *Torula* species was transmitted by some insect carrier. This was suggested by the very extensive work of Steinhaus (1941), whose study of the bacteria associated with many species of insects has given great emphasis to the source of this contamination. In his classic contribution to this subject he showed that from the 30 species of insects studied he isolated 83 strains of bacteria. Since the one insect which is almost ubiquitous in the Temperate Zone is the cockroach, it would have been first in our list of suspects in this connection. This suspicion was given added weight by the fact that the only two instances where Steinhaus isolated yeasts from insects were from those species of the order of *orthoptera,* in one case the genus *Lactilla* and in the other the genus *Diapheromera.* While a comparison of the morphological characteristics of the yeasts isolated by Steinhaus and the *Torula* which we had been studying revealed that the species were not identical, the fact that the roach seems to be a definite carrier of yeast encouraged the belief that this insect might be the habitat of the organism sought.

In endeavoring to isolate *Torula acidophila* from roaches, it was, of course, necessary to collect them from points remote from areas in which the sirup under study was exposed; otherwise the insect might come in contact with the contaminated material and might serve as a carrier rather than the original habitat of the organism. The first line of approach was in exposing sterilized, refined sugars in sterile dishes in cages where various species of roaches were confined. The sugar being the principal source of food, it would necessarily be subject to unavoidable contamination and it was thought that in feeding and in incidental contact with the sugar that the contaminant for which we were seeking might be transferred to the sugar crystal. After an exposure of this kind for a week or 10 days the sugar was brought to our laboratory, cultured in the usual way, and plated upon various agar substrates. Later, this method was modified and the sugar was acidified to a pH corresponding to the sirup under investigation and incubated for several days before being subjected to bacteriological analysis. The advantages of this procedure were that it eliminated bacterial species and also screened out all yeasts lacking the same degree of tolerance of acidity as that of the species which we were seeking. Since the exposed sugar samples were reduced to approximately the concentration of the sirup and acidified water was added, the appearance of fermentation in these samples was almost proof of the presence of *Torula acidophila* or some very closely related species of yeast. Although we encountered many instances in which some slight fermentation occurred, and some small yeasts were isolated, we were not able by this means to obtain a species corresponding to *Torula acidophila.*

The foregoing procedure was followed by the method of exposing sugars, as in the first case, to various species of roaches; taking the insects and sterilizing their surfaces with alcohol, according to the technique originated by Steinhaus; and crushing the insects in sterile tubes and adding enrichment medium directly to the insects so prepared. Exposing insects to sugar prior to their maceration and examining their abdominal tracts was for the purpose of furnishing a suitable carbohydrate for the growth of yeast in their digestive tracts. In every case, control samples of the sterile sugar were held and these samples were treated with enrichment media to make certain that the organism in question was not originally present in the material exposed. This may have been somewhat superfluous inasmuch as a four-year study of sugars had not revealed this particular species of *Torula*. The following species of roaches were supplied by the Department of Entomology of the Louisiana State University, which kindly co-operated with us in this investigation: the American cockroach, *Periplaneta americana* (Linn.) and the German cockroach, *Blatella germana* (Linn.).

From several hundred male and female specimens of both the American and German cockroach, we were successful in obtaining cultures of *Torula acidophila* from a large number of the American species but were never successful in isolating it from the German cockroach. This does not, by any means, disprove the possibility of the latter species being a carrier of this particular organism for, so far as we are aware, there is no particular difference in the feeding habits of the two species. The difference may be that the American cockroach is much more migratory than the German.

While a search for the presence of *Torula acidophila* was applied both to the surface area and intestinal tracts of these insects, the organism was found to occur far more frequently within than upon them. It would seem, therefore, that the normal habitat of this yeast is within the digestive tract of the cockroach and that when it occurs on the surface, it is the result of contamination from the former source. It is reasonable to believe that this species of yeast finds the abdominal tract of certain species of cockroaches highly favorable for its development, especially when fermentable carbohydrates are used as food by the insect in question. In fact, the direct microscopical examination of the contents of the digestive tract of the American cockroach almost always reveals the presence of yeast which conform, in their morphological characteristics, to *Torula acidophila*.

This organism imparts a characteristic flavor, suggestive of malic acid, to sirups in which it occurs. In fact, the flavor is so distinctive that the experienced observer can almost identify the presence of the organism by the characteristic taste. The malic acid flavor is the chief cause of the deterioration induced by *Torula acidophila* since it superimposes a foreign taste upon the original flavor of the sirup. While we were unable to identify the source of this flavor, it is unquestionably an ester of a rather peculiar type.

SURVIVAL OF ORGANISM IN A DRY STATE

Upon the assumption that *Torula acidophila* is subjected, at times, to desiccation either in dust or in contaminated sugars, or in desiccated

digestive tracts of dead insects, tests were made on the survival rate of pure cultures of *Torula* when disbursed on products and subjected to various degrees of desiccation. The materials used in these tests included paper, soil, sugar, sirup, gelatin, and activated carbon. It was found that when refined sugars were seeded with *Torula acidophila* and dried to the normal moisture content of sugar, namely 0 to 0.835 per cent, the organism remained viable for only one week. It is not readily conceivable that it could survive long periods in ordinary commercially refined sugars. On the soil, the survival was almost one month. The results of some of these drying tests are given (Table 3).

TABLE 3

Results of Some Drying Tests on Survival Rate of Pure Cultures of Torula

Material	Macro and micro examination [1]			
	Bacteria	Molds	Yeast	*Torula*
Paper sugar bags	+	+	—	—
Cotton sugar bags	+++	+++	—	—
Extract of coco	++++	+	+	+?
Sirup with caramel	++++	—	+	—

[1] + = present; ++ = present in small numbers; +++ = present in large numbers; and ++++ = very high percentage of contamination.

In sirups, however, we have found that *Torula* can survive for years since the products of its metabolism do not seem to reach levels that are very harmful to the organism.

Further tests were made of the resistance of this organism to drying, by impregnating various substances with viable cells and drying these by various means at atmospheric temperatures (Table 4).

There are two other characteristics of this *Torula* which make it of special interest. The first is its very low requirement for nitrogenous nutrients since the sirup in which it occurs rarely contains more than 30 to 35 milligrams of nitrogen per 100 cubic centimeters. The addition of as little as 0.1 c.c. of 0.5 per cent peptone solution per 100 c.c. of sirup greatly accelerated the rate of fermentation. For this reason sirups made from highly refined sugars with no other addition of nitrogen may be highly susceptible to the growth of this species of yeast.

The second distinctive feature of this yeast is its ability to grow in the presence of surface depressants. Because of the essential oil contained in the sirups in which this organism occurs the surface tension is slightly depressed, the values varying from approximately 67.59 to 72.9 dynes. According to Kisch (1912), *Saccharomyces cerevisiae* is much more sensitive to surface depressants than are bacteria; he also found that the former is permanently injured at surface tensions of less than 36. In view of the fact that *Torula acidophila* can grow within a pH of 1.5 to 7 and considering that the depressed surface tension does not inhibit its development, the thought is suggested that these characteristics would naturally endow the organism with the ability to develop in the human digestive tract; therefore the determination of its resistance to the presence of bile salts, which are used as a standard practice in the determination of enteric

organisms, seemed very appropriate. With this in view, the following experiment was conducted and a substrate of the following composition was employed:

Bacto peptone	10.0 grams
Bacto ox gall	50.0 grams
Bacto dextrose	10.0 grams
Water	1,000.0 c.c.
pH	7.25

This medium was inoculated with *Torula acidophila* and incubated at 34°C.(93.2°F.). After five days there was an abundant growth, compact sediment, and cells of the organism were abundant and normal in contour and dimensions.

TABLE 4

Results of Further Tests for Resistance of Torula acidophila to Drying

Method of drying	Carrier of organism	Material used	Total days dried	State	Checking		Notes
					Viable days	Dead days	
Air	Broth	Paper	64	Dry	11	22	.....
Air	Broth	None	64	Dry	22	37	.....
H_2SO_4	Broth	None	48	Dry	48	53	Molded
$CaCl_2$	Broth	None	53	Dry	16	31	Molded
Air	Water	Soil	29	Dry	17	29	Molded
$CaCl_2$	Water	Soil	29	Dry	27	33	.....
Air	Sirup	Sugar	30	Dry	17	15	.....
$CaCl_2$	Sirup	Sugar	30	Dry	7	7	.....
Air	Sirup	Sugar	28	Dry	?	7	.....
$CaCl_2$	Sirup	Sugar	28	Dry	?	7	.....
Air	Water	Gelatin	30	Wet	30	....	.....
$CaCl_2$	Water	Gelatin	30	Wet	30	....	.....
Air	Water	Filter cel	28	Dry	28	....	.....
$CaCl_2$	Water	Filter cel	28	Dry	?	....	Molded
Air	Water	Carbon	30	Wet	30	....	Molded
$CaCl_2$	Water	Carbon	30	Dry	?	....	Molded
Air	Sirup	Sugar sirup	30	Liquid	30	....	.....
$CaCl_2$	Sirup	Sugar sirup	30	Liquid	30	....	.....

The above medium contains five per cent of ox gall, as contrasted with the usual two per cent formula for an enrichment medium for the determination of the coliform organism in water. It was found, upon tests, that *Torula acidophila* grows vigorously in the above medium, indicating its ability to survive in the human digestive tract. This is a very significant property on the part of this species of yeast.

PATHOGENICITY OF YEAST

The fact that the roach seems to be the principal vector of this new species of yeast is significant in that many of the pathogenic species of yeast recorded in the literature are also transmitted by insects. For example, the yeast discovered by Btuschilii and Dangeard, which is pathogenic for animals and insects, has as its habitat the intestines of *Culex*

(mosquitoes) and that discovered by Lindner, the larvae of the fly. In view of the low pH tolerance of this yeast and its source it might be regarded as a potential causative agent of enteric disorders, and this possibility seems to warrant further investigations.

ACKNOWLEDGMENT

The writers are indebted to the authors of "The Yeasts" (Guilliermond and Tanner) for their extensive description of the saccharomycetes, to which frequent reference was made both as to subject matter and bibliography in preparing this article.

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QUALITY AND PALATABILITY OF BEEF AS AFFECTED BY METHOD OF FEEDING AND CARCASS GRADE [1]

J. J. WANDERSTOCK AND J. I. MILLER
Department of Animal Husbandry, Cornell University, Ithaca, New York

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Beef consumers have long been interested in the quality and palatability of beef from carcasses of various grades. To obtain data on factors which might be affected, studies were undertaken on beef produced by yearling steers fed and managed according to methods ranging from entire dry-lot feeding to fattening solely on pasture. Various combinations of these two extremes were also investigated.

Hirzel (1939), Hammond (1940), and Hankins (1945), reviewed the literature relative to factors influencing carcass quality in beef. Black, Hiner, Burk, Alexander, and Wilson (1940); Bull, Snapp, and Rusk (1941); and others indicated that beef produced by animals fattened on grass alone graded lower and was slightly lower in palatability than that produced by animals receiving supplemental concentrates. Branaman, Hankins, and Alexander (1936) indicated that, within reasonable limits, the fatter an animal is the higher will be the dressing percentage, carcass grade, and percentage of fat in the carcass, and the lower will be the percentages of moisture, protein, lean, and bone.

Yellow fat and dark lean lower the market grade of beef. Bull, Snapp, and Rusk (1941) and Hankins and Barbella (1941) demonstrated a greater yellowness of fat associated with cattle fattened on grass as compared with dry-lot-fed cattle. Hall, Latschar, and Mackintosh (1944) indicated that the most significant difference in the properties of dark beef and bright beef appeared to be the lower amounts of lactic acid in the former. One of the usual characteristics of dark-cutting beef was an abnormally high pH.

From the standpoint of the discriminating consumer, a knowledge of the nature of the various cuts of beef, especially their proportions of lean meat, fat, and bone, is an absolute necessity. Black, Hiner, Burk, Alexander, and Wilson (1940) and Hankins and Foster (1940) showed that the feeding of concentrates in addition to grass had a marked effect, in general, on increasing the proportion of meat to bone. An extensive experiment conducted by Wilson and Company (1943), in co-operation with the United States Department of Agriculture and the University of Illinois, indicated that the higher the carcass grade, the greater the dressing percentage and percentage of fat and the lower the percentages of lean and bone. Hopper (1944) and Hankins and Howe (1946) proposed methods for estimating the physical and chemical composition of a beef carcass.

[1] Includes part of thesis submitted by J. J. Wanderstock in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Cornell University.

The ultimate test of the acceptability of beef is its palatability. The palatability data of Black, Warner, and Wilson (1931); Black, Hiner, Burk, Alexander, and Wilson (1940); and Bull and Rusk (1942) indicated rather slight differences between roasts from cattle full-fed in dry lot as compared with those from cattle full-fed on pasture or fattened on grass alone; however, grade differences were also slight.

OBJECTIVES

The purpose of these studies was to compare the quality and palatability of various grades of beef produced by yearling steers of known feeding and management history. It was desired to determine the degree of fatness, as represented by the carcass grade, necessary to produce acceptable beef.

BEEF USED IN EXPERIMENTS

The beef studied in these experiments was produced in a feeding and management experiment conducted to determine the feeding requirements and outcome of yearling steers fattened on pasture alone, by grain feeding on pasture, following pasture, or in dry lot.

Starting in the summer of 1943, four uniform lots of 10 good-to-choice yearling Hereford steers, purchased in the spring from Texas, were fed and managed as follows: Lot I, full-fed in dry lot a ration of coarsely ground corn, protein supplement (equal parts linseed meal and soybean oil meal), and mixed grass and legume hay (mostly timothy); Lot II, grazed on improved bluegrass and white clover pasture, and full-fed coarsely ground corn in addition to grass; Lot III, grazed on good improved pasture during the early pasture season, then full-fed in dry lot the same ration as Lot I; Lot IV, grazed on the same pasture as Lot III for the entire experiment, receiving no grain or protein supplement whatsoever (this experiment was repeated in 1944); Lot V, grazed for the entire pasture season, then full-fed in dry lot the same ration as Lot III, was substituted for Lot I. The third series of feeding trials, duplicating those of 1944, was conducted in 1945.

A brief summary of these feeding trials, reported by Miller, Morrison, and Tabet (1944) and Miller, Morrison, Briggs, and Blaser (1947), is presented (Table 1).

EXPERIMENTAL PROCEDURE

In the first two experiments, five representative steers, and in the third trial, four representative steers, were selected from each lot of 10 steers for the meat studies. All animals were slaughtered according to standard methods. The carcasses were immediately chilled and allowed to hang in a meat cooler maintained at 0 to 2.2°C.(32 to 36°F.) for 10 days (13 days in the 1943 experiment) before any detailed studies were undertaken. Then they were divided into standard wholesale cuts.

The right rib cut was boned and rolled into four- to five-pound roasts, which were frozen for subsequent use in palatability studies (in 1943 corresponding fresh roasts were also used). The left rib cut was used for (a) color studies of lean and fat, determined by the Munsell disk-spinning method; (b) the determination of the area of the rib-eye muscle, using a compensating polar planimeter; (c) the physical separation of ribs 9-10-11,

TABLE 1

Summary of Feeding Trials

Weight and grade	Lot I—full-fed in dry lot	Lot II—full-fed on pasture			Lot III—pasture-fed, then full-fed, in dry lot			Lot IV—pasture only			Lot V—pasture-fed entire season, then full-fed in dry lot	
	1943	1943	1944	1945	1943	1944	1945	1943	1944	1945	1944	1945
Av. initial weight (lb.)	635	633	566	713	635	562	702	630	553	702	553	691
Av. final weight (lb.)	866	869	802	1036	890	815	1002	811	692	903	853	1073
Av. daily gain (lb.)	2.05	2.11	1.96	2.15	1.66	1.51	1.63	1.38	1.06	1.40	1.17	1.57
Grade on foot, end of trial [1]	2.7	3.5	3.8	2.5	3.6	5.5	4.8	6.2	9.5	7.6	5.3	4.1
Federal carcass grade [1]	2.6	3.5	4.2	3.2	3.8	5.2	6.2	6.0	8.9	8.3	5.1	4.2
Av. dressing percentage	58.3	57.4	57.1	60.8	57.1	55.8	58.6	53.1	51.8	53.6	57.8	58.3

[1] The final grades on foot and the carcass grades are based on giving the following numbers and taking an average for each lot; hence the higher the number, the lower the grade.

Top choice....1	Top good....4	Top medium....7	Top common....10
Average choice....2	Average good....5	Average medium....8	Average common....11
Low choice....3	Low good....6	Low medium....9	Low common....12

10 inches long, into lean, fat, and bone; (d) press-fluid studies of a sample of the longissimus dorsi muscle, using a Carver hydraulic laboratory press; and (e) the determination of the pH and moisture content of this same muscle.

Each carcass was federally graded. Shrinkage and dressing percentages were calculated. Moisture content was determined by A.O.A.C. vacuum-oven method [six hours at 90°C.(194°F.) *in vacuo*] in an atmosphere of carbon dioxide. The pH was determined by the glass-electrode method.

Cooking and palatability studies were conducted under controlled conditions in an experimental kitchen of the New York State College of Home Economics at Cornell University. The roasts to be frozen were wrapped in moisture-vapor-proof cellophane paper and in stockinette, placed in a home freezer at —22.2°C.(—8°F.), and stored therein at —17.8°C.(0°F.). Storage was for not longer than three months in the first experiment and six months in the other two trials.

Before cooking, the roasts were thawed in their own wrappings for 48 hours in a household refrigerator. They were cooked to an internal temperature of 60°C.(140°F.). Drip and evaporation during cooking were determined. Six or seven experienced judges assisted in each "blind" test, scoring the roasts according to the chart developed by the National Research Project, "Cooperative Meat Investigations."

RESULTS

Dressing Percentages and Carcass Grades: The steers fattened on pasture alone, without any grain or supplements (Lot IV), had significantly lower final grades on foot in each experiment than did those of any of the other lots. The differences between the other lots in final grade on foot were small.

After slaughter, the carcasses were promptly chilled to below 4.4°C. (40°F.). Shrinkage from warm- to cold-carcass weights averaged about two per cent. Dressing percentages were calculated on the basis of these cold-carcass weights (Table 1). The steers in Lot IV, in each experiment, had significantly lower dressing percentages and carcass grades than did those of any of the other lots. The range in carcass grade for the various lots was low to average choice for Lot I, top good to low choice for Lot II, low good to low choice for Lot III, low commercial to low good for Lot IV, and average to top good for Lot V.

Color Studies of Lean and Fat: Samples of external fat and of the rib-eye muscle were studied with a Munsell disk-spinning apparatus for differences in color. These samples were compared with four adjustable standard disks. Computations for the Munsell values, Hue (distinctive primary characteristic of any chromatic color, e.g., red, yellow, etc.), Value (lightness or darkness, varying from the blackest black, 0, to the whitest white, 10), and Chroma (strength or weakness), were made according to the method suggested by Newhall, Nickerson, and Judd (1943).

The averages of the individual observations for color of lean and external fat (Table 2) indicate that in the 1943 experiment the lean from the carcasses in Lot I, the highest grading lot, was redder than that of any other lot. It was bright red, lightened by interspersed fat particles (mar-

bling). The fat also had a reddish tinge in contrast to the yellowish cast of the fat samples from the other lots. The differences in the Hue of lean were slight, ranging from 6.8R in Lot I to 7.2R in Lot II. The most extreme differences in the Hue of external fat were between Lots I and IV, 6.7YR and 8.7YR, respectively, Lots II, III, and IV being very similar. Differences in Value and Chroma were likewise small.

TABLE 2

Color Studies of Lean and Fat

Lot No. and year	Lean			Fat		
	Hue	Value	Chroma	Hue	Value	Chroma
Lot I—Full-fed in dry lot						
1943	6.6R	3.9	4.9	6.7YR	7.3	2.8
Lot II—Full-fed on pasture						
1943	7.2R	3.7	5.1	8.6YR	7.3	3.6
1944	7.0R	4.1	4.3	7.5YR	7.2	3.4
Lot III—Pasture-fed, then full-fed in dry lot						
1943	7.1R	3.6	5.1	8.3YR	7.6	2.3
1944	7.4R	4.0	4.8	8.0YR	7.4	2.9
Lot IV—Pasture only						
1943	6.9R	3.9	4.7	8.7YR	7.1	3.2
1944	7.4R	3.9	4.6	8.7YR	7.1	3.8
Lot V—Pasture-fed entire season, then full-fed in dry lot						
1944	7.0R	3.8	4.8	6.8YR	6.9	3.7

In 1944, the averages of the individual observations for color of lean indicated little difference between lots in all three attributes. Differences in color of fat showed greater variation. The degree of yellowness was least in the fat from Lot V and increased in Lots II, III, and IV, in that order.

Because of the slight differences in color of both lean and fat, as measured by this method, color was not determined in the 1945 experiment.

The grading of the carcasses was not influenced by the color of fat. The yellowness was noticeable to the eye, but no significant differences were measured by the method used. In the opinion of the authors, an improvement is necessary in the technique of estimating color of fat.

Moisture and pH Determinations: The average moisture and pH values are shown (Table 3); moisture determinations of a sample of the rib-eye muscle indicated that the fatter the carcass and the greater its degree of marbling, the smaller the percentage of moisture. Thus, each year, there was a slight trend for higher moisture content in the lower grading carcasses.

Since some workers reported a relationship between pH and dark-cutting beef, the higher pH values being characteristic of dark cutters, it was desired to determine the relationship, if any, between color of lean and pH. The samples of the rib-eye muscle analyzed for pH gave values ranging from 5.42 to 5.80, which were within the normal range for beef. The differences were not associated with the color of lean; therefore, under the conditions of these investigations and within the

relatively small range in pH, there was no marked correlation between color of lean and pH.

Press-Fluid Determinations: To determine objectively the juices of the lean, a 100-gram sample of the fresh rib-eye muscle was taken and the juice was expressed at a pressure of 4,000 pounds per square inch, using a Carver hydraulic laboratory press. The range in the measured amount of juice was from 35 to 49 c.c. in the 1943 trial and from 36 to 52 c.c. in the 1944 experiment. The averages for the various lots indicated that, in general, the more finish a carcass carried the smaller was the measured amount of expressible juice (Table 3). Apparently, fat particles tend to inhibit the loss of press fluid. Loss by evaporation in this determination did not exceed 10 per cent. There was an inverse relationship between the loss by evaporation and the amount of press fluid. Therefore, beef having a higher degree of finish and a lesser amount of expressible juice should be juicier when cooked.

TABLE 3

Moisture and pH Determinations; Press-Fluid Studies

Lot No. and year	Moisture	pH	Measured amount of juice	Weight pressed meat	Loss by evaporation
	pct.		*c.c.*	*gm.*	*pct.*
Lot I—Full-fed in dry lot[1]					
1943	74.0	5.5	39.6	50.4	10.0
Lot II—Full-fed on pasture[2]					
1943	75.4	5.4	45.2	45.2	9.6
1944	75.2	5.6	44.6	47.0	8.4
1945	74.4	.....	.....	.....	.....
Lot III—Pasture-fed, then full-fed in dry lot[3]					
1943	75.7	5.6	46.4	44.6	9.4
1944	75.2	5.6	45.0	46.6	8.4
1945	74.9	.....	.....	.....	.....
Lot IV—Pasture only[4]					
1943	76.3	5.5	46.2	44.5	9.3
1944	76.3	5.6	48.6	43.7	7.7
1945	75.2	.....	.....	.....	.....
Lot V—Pasture-fed entire season, then full-fed in dry lot[5]					
1944	74.5	5.8	45.4	46.2	8.4
1945	74.7	.....	.....	.....	.....

[1] Carcass grades low to average choice. [2] Carcass grades top good to low choice. [3] Carcass grades low good to low choice. [4] Carcass grades low commercial to low good. [5] Carcass grades average to top good.

Area of Rib-Eye Muscle: Averages of individual observations of the area of rib-eye muscle varied slightly between lots in 1943; the difference between Lot IV (average commercial grade) and any of the other lots was highly significant in 1944 (Table 3). In each of these experiments the correlation between area of rib-eye muscle and cold-carcass weight was highly significant, indicating that the area of rib-eye muscle varied directly with the weight of the carcass.

Physical Analyses of Left Ribs 9-10-11: Left ribs 9-10-11 were separated into eye muscle, other lean, external fat, other fat, gristle, and bone to study the comparative development of the steers in these characteris-

TABLE 4

Physical Analyses of Left 9-10-11 Rib Cut [1]

Grade and component	Lot I—full-fed in dry lot	Lot II—full-fed on pasture			Lot III—pasture-fed, then full-fed, in dry lot			Lot IV—pasture only			Lot V—pasture-fed, entire season, then full-fed in dry lot	
	1943	1943	1944	1945	1943	1944	1945	1943	1944	1945	1944	1945
Carcass grades	Low to average choice	Top good to low choice			Low good to low choice			Low commercial to low good			Average to top good	
Eye muscle	18.3	20.5	19.9	21.1	21.2	22.3	21.3	24.4	23.4	25.3	22.5	21.3
Total lean	47.1	53.5	54.5	50.4	51.9	53.8	51.6	60.5	60.0	57.9	54.2	50.8
External fat	12.5	10.2	9.2	13.0	10.4	10.0	8.8	4.8	3.8	7.5	10.3	11.7
Total fat	35.4	27.0	26.6	32.6	28.1	26.1	29.0	16.2	14.1	22.4	28.2	32.8
Total edible portion	82.5	80.5	81.1	83.0	80.0	80.0	80.7	76.7	74.2	80.2	82.4	83.6
Bone, including gristle	17.5	19.5	18.9	17.0	20.0	20.0	19.3	23.3	25.8	19.8	17.6	16.4
Ratio of edible meat to bone	4.7:1	4.1:1	4.3:1	4.9:1	4.0:1	4.0:1	4.2:1	3.3:1	2.9:1	4.1:1	4.7:1	5.1:1
Area of rib-eye muscle (sq. in.)	8.65	9.21	8.40	.....	9.03	8.56	.....	7.92	6.75	.....	8.59	.....

[1] All figures in per cent, except ratio of edible meat to bone and area of rib-eye muscle.

tics. The percentages of these components were calculated, using the actual weights (Table 4).

In the 1943 trial, for all factors studied, the carcasses from Lot I were at one extreme and those from Lot IV, the pasture-only group, were at the other extreme in per cent composition, while the carcasses from Lots II and III were similar but more nearly like Lot I than Lot IV. In the 1944 and 1945 trials the carcasses from Lot V were at one extreme and those from Lot IV were at the other extreme in per cent composition. Those from Lot II were more similar to Lot V in all characteristics, while those from Lot III were more similar to Lot V in total lean and total fat.

In each trial the carcasses from Lot IV averaged highest in per cent eye muscle, per cent total lean, and per cent bone (including gristle), and lowest in per cent external fat, per cent total fat, and per cent total edible portion (the sum of eye muscle, other lean, external fat, and other fat).

From these data the ratio of total edible meat to bone (including gristle) was calculated. This ratio was widest for Lot I (the low-to-average-choice carcasses) in the first trial and for Lot V (the average-to-top-good carcasses) in the other two trials, indicating a relatively high proportion of meat to bone. In all three experiments this ratio was narrowest for Lot IV (the commercial carcasses) and significantly narrower in 1943 and 1944, indicating a relatively low proportion of meat to bone.

Thus, the data obtained in the physical analyses indicated that under the conditions of these investigations the addition of grain to the ration for yearling steers improved the grade of beef and thereby resulted in a greater proportion of meat to bone than was evident in the case of the carcasses of steers fed grass only.

Cooking and Palatability Studies: Cooking and palatability studies were conducted on rolled rib roasts representative of each carcass (Table 5).

In the 1943 trial two roasts from each carcass were used, one fresh and the other frozen. Since all phases of the experiments did not end at the same time, it was desirable to freeze roasts to use them subsequently in comparisons when a roast from each lot would be available. It was also considered expedient to cook fresh roasts as a check on the frozen roasts. The cooking losses and average palatability scores (desirability) of the fresh and frozen roasts are shown (Table 6).

The total cooking losses of the fresh roasts varied slightly between lots, drippings being highest in those from Lot I and lowest in those from Lot IV. Evaporation loss, in general, varied inversely to loss in drippings. Palatability scores were highest for the roasts from Lot I and lowest for those from Lot IV. The roasts from Lots II and III were similar, and most like those from Lot I. The principal palatability differences (desirability) among the roasts occurred in aroma, flavor of fat, flavor of lean, quality of juice, and juiciness. In these factors, as well as in average palatability score and score for over-all appearance, the roasts from Lot IV were significantly poorer than were those from any of the other lots. All of the roasts were considered acceptable.

The total cooking losses of the fresh roasts in the 1943 trial also varied slightly between lots, and the palatability scores closely paralleled those of the fresh roasts.

TABLE 5

Cooking Losses and Average Palatability Scores (Desirability) for Frozen Rib Roasts

Grade, cooking loss, palatability score	Lot I—full-fed in dry lot	Lot II—full-fed on pasture			Lot III—pasture-fed, then full-fed in dry lot			Lot IV—pasture only			Lot V—pasture-fed entire season, then full-fed in dry lot	
	1943	1943	1944	1945	1943	1944	1945	1943	1944	1945	1944	1945
Carcass grades	Low to average choice	Top good to low choice			Low good to low choice			Low commercial to low good			Average to top good	
Cooking losses[1]												
Drippings												
Not fat	1.3	2.5	1.8	1.6	2.5	2.0	2.0	3.2	3.2	1.9	1.2	1.6
Total	4.9	4.6	3.5	4.5	5.0	3.2	4.3	3.9	3.3	3.6	3.5	4.8
Evaporation	15.7	19.4	8.5	12.2	17.9	8.6	12.9	18.9	9.5	11.9	9.0	12.4
Total	20.6	24.1	12.0	16.7	22.9	11.8	17.1	22.8	12.8	15.5	12.5	17.3
Palatability scores[2]												
Aroma	6.3	5.8	5.2	5.6	6.0	5.1	5.5	3.7	4.8	5.2	5.7	5.8
Texture	6.0	5.8	5.8	5.7	5.9	5.7	5.5	5.5	5.7	5.5	5.7	6.0
Flavor of fat	6.2	5.6	5.0	5.2	5.7	4.8	5.4	4.4	3.8	4.7	5.7	5.8
Flavor of lean	6.5	5.6	5.5	5.8	6.0	5.3	5.6	4.3	3.7	5.2	5.9	5.9
Tenderness	6.5	6.0	5.4	5.9	5.8	5.6	6.0	5.2	4.3	5.9	5.9	5.7
Quality of juice	6.2	5.6	5.9	5.8	5.9	5.6	6.0	3.7	4.5	5.3	6.1	5.9
Juiciness	6.1	5.1	5.9	5.8	5.2	5.6	5.5	4.3	3.8	5.1	6.4	5.8
Average	6.2	5.6	5.5	5.7	5.8	5.4	5.7	4.4	4.4	5.3	5.9	5.8
Average over-all judgment[3]	4.7	4.5	4.2	4.6	4.5	4.0	4.1	3.9	2.7	3.9	4.8	4.6

[1] Cooking losses in per cent. [2] Palatability scores are average of six or seven judges, and range from 0 to 7 with increasing desirability. [3] Averages of six or seven judges, and range from 0 to 5 with increasing desirability.

TABLE 6

Cooking Losses and Average Palatability (Desirability) Scores for Fresh and Frozen Rib Roasts, 1943

Cooking loss and palatability score	Lot I—full-fed in dry lot			Lot II—full-fed on pasture			Lot III—pasture-fed, then full-fed in dry lot			Lot IV—pasture only			Average		
	Fresh	Froz.	Diff.	Fresh	Froz.	Diff.	Fresh	Froz.	Diff.	Fresh	Froz.	Diff.	Fresh	Froz.	Diff.
Cooking losses[1]															
Drippings															
Not fat	0.7	1.3	0.6	1.1	2.5	1.4	1.0	2.5	1.5	2.0	3.2	1.2	1.2	2.4	1.2
Total	4.6	4.9	0.3	3.8	4.6	0.8	3.3	5.0	1.7	2.6	3.9	1.3	3.6	4.6	1.0
Evaporation	9.1	15.7	6.6	10.0	19.4	9.4	8.7	17.9	9.2	10.1	18.9	8.8	9.5	18.0	8.5
Total	13.7	20.6	6.9	13.8	24.1	10.2	12.0	22.9	10.9	12.7	22.8	10.1	13.1	22.6	9.5
Palatability scores[2]															
Aroma	6.4	6.3	—0.1	6.2	5.8	—0.4	5.6	6.0	0.4	4.4	3.7	—0.7	5.7	5.5	—0.2
Texture	5.7	6.0	0.3	5.2	5.8	0.6	5.4	5.9	0.5	5.2	5.5	0.3	5.4	5.8	0.4
Flavor of fat	6.3	6.2	—0.1	5.4	5.6	0.2	5.3	5.7	0.4	3.4	4.4	1.0	5.1	5.5	0.4
Flavor of lean	6.5	6.5	.....	6.2	5.6	—0.6	6.0	6.0	.....	5.0	4.3	—0.7	5.9	5.6	—0.3
Tenderness	6.4	6.5	0.1	5.6	6.0	0.4	5.9	5.8	—0.1	5.6	5.2	—0.4	5.9	5.9	.....
Quality of juice	6.5	6.2	—0.3	6.1	5.6	—0.5	6.3	5.9	—0.4	3.8	3.7	—0.1	5.7	5.4	—0.3
Juiciness	6.3	6.1	—0.2	5.9	5.1	—0.8	5.8	5.2	—0.6	5.0	4.3	—0.7	5.8	5.2	—0.6
Average	6.3	6.2	—0.1	5.8	5.6	—0.2	5.8	5.8	.....	4.6	4.4	—0.2	5.6	5.5	—0.1
Average over-all judgment[3]	4.8	4.9	0.1	4.5	4.5	.....	4.9	4.6	—0.3	3.2	3.7	0.5	4.4	4.4	.....

[1] Cooking losses in per cent. [2] Palatability scores are averages of six or seven judges, and range from 0 to 7 with increasing desirability. [3] Averages of six or seven judges, and range from 0 to 5 with increasing desirability.

In comparing the results obtained with the fresh and frozen roasts, marked differences were noted (Table 6). A longer period of time per pound was required in cooking roasts which had been frozen than was required for the fresh roasts, probably owing to the lower average temperature of the former when placed in the oven. A greater per cent of total drippings, drippings exclusive of fat, evaporation, and total cooking losses occurred in the case of the frozen roasts. Average total drippings from these roasts were 27.8 per cent higher, drippings exclusive of fat were 100 per cent higher, evaporation was 89.5 per cent higher, and total cooking loss was 72.5 per cent higher than comparable losses of the fresh roasts. These decidedly greater losses from the frozen roasts were probably due to the longer cooking time required, as well as to histological changes (rupture of cells) during freezing and thawing. The average palatability scores for the frozen roasts were slightly lower than for the corresponding fresh roasts but in the same relative position. The differences between the scores for the fresh and frozen roasts for the various factors were slight.

In 1944 and 1945, the average per cent total cooking losses varied only slightly between lots. These losses were not as great as were those, also obtained with frozen roasts, in 1943. The average per cent drippings, exclusive of fat, was lowest in the roasts from Lot V. In 1944, the major palatability differences were flavor of fat, flavor of lean, tenderness, quality of juice, and juiciness. The roasts from Lot V ranked highest in almost all of these factors. Those from Lot IV, the grass-only group, which graded low commercial, were significantly poorer in these palatability factors than were the roasts from any of the other lots. In the 1945 trial the roasts from Lot IV were lowest in all palatability factors with the exception of tenderness. The roasts from Lot V ranked slightly higher in most factors than did those from Lots II and III. In 1945, the differences in palatability were less marked than in the previous experiments.

There was an inverse relationship between per cent drippings exclusive of fat, and the following: average palatability score, average palatability score (excluding intensity, except for texture and tenderness), flavor of lean, tenderness, and juiciness. These correlations were significant in the first two experiments.

The three trials indicated that beef produced by feeding concentrates in addition to pasture or in dry lot, largely because of increased fatness which resulted in carcass grades ranging from low good to average choice, was definitely superior to that produced by fattening on pasture alone which resulted in low-commercial to low-good carcasses. Under the conditions of these investigations with yearling steers, pasture alone did not produce beef of high enough quality to compete with that produced by grain feeding.

SUMMARY

Carcasses of representative yearling steers from three feeding trials comparing beef produced by fattening on pasture alone with that produced by feeding grain on pasture, after pasture, or in dry lot were used to obtain data on the following: final grade on foot, dressing percentage, carcass grade, color of lean and fat, moisture and pH content, press fluid, area of rib-eye muscle, physical analysis of left ribs 9-10-11, cooking losses, and palatability scores.

Each year the steers receiving no grain or other supplements had significantly lower final grades on foot, dressing percentages, and carcass grades than did those of any of the other lots.

The averages of the individual observations for color of lean and external fat showed slight differences between lots. This also occurred in moisture and pH content and in press fluid.

The physical analyses of left ribs 9-10-11 indicated that the pasture-only carcasses, which graded average commercial, had the smallest proportion of meat to bone. The correlation between the area of the rib-eye muscle and cold-carcass weight, in all lots, was highly significant.

Cooking losses varied only slightly between lots. In the first experiment, when both fresh and frozen roasts were used, these losses were decidedly greater in the latter. The roasts from the pasture-only carcasses, which had an average grade of commercial, were significantly poorer in palatability and in over-all appearance than were those from the concentrate-fed groups. The differences were less marked in the last trial when the pasture season was unusually good and heavier cattle were used at the start.

Under the conditions of these three trials, all of the beef was acceptable; however, largely owing to a difference in fatness, beef produced by feeding grain on pasture (grading top good to low choice), after pasture (grading low good to low choice), or in dry lot (grading low to average choice) was higher in quality and palatability than that produced by fattening on pasture alone with no grain or supplements (grading low commercial to low good).

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INFLUENCE OF LIGHT DURING STORAGE ON COMPOSITION OF BLUE HUBBARD SQUASH[1]

ARTHUR D. HOLMES, ALBERT F. SPELMAN, CHARLES J. ROGERS, AND WILLIAM H. LACHMAN
Massachusetts Agricultural Experiment Station, Amherst, Massachusetts

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An earlier study of the composition of squashes after winter storage showed that they were good sources of food ingredients essential for the human diet. The squashes which were used in that study were procured from a variety of sources with the hope that the results would be applicable to market squashes produced in the New England area. The values for the nutrients studied, however, varied not only with the different varieties but also considerably with the individual squashes within the varieties. Thus a question arose regarding the possible influence of storage conditions upon the composition of squashes at the end of winter storage. This was particularly interesting with respect to light, for in this area squashes may be stored in greenhouses fully exposed to bright daylight or they may be stored during the winter months in the dark in specially constructed squash houses. The present study was arranged to collect data regarding the possible influence of light, during winter storage, on the composition of squashes.

EXPERIMENTAL PROCEDURE

Since Blue Hubbard squash is extensively grown in this area and is typical of the hard-shelled varieties of winter squashes, it was chosen for this study. The squash seed, which was procured from a commercial seed house, was planted the second week in June in a Merrimac fine sandy loam soil which has been previously described by Holmes (1944). A commercial 5-10-5 fertilizer was applied at the rate of 1,500 pounds per acre. During the early growing period the squash plants were dusted with rotenone to prevent insect infestation. The squash plants received about 1,100 hours of bright sunshine and 16 inches of rain during the growing season and the temperature ranged between 35°C.(95°F.) in July and 0°C.(32°F.) in October. The squashes were harvested during the first week of October and stored for two months in a greenhouse in order to eliminate the squashes that were likely to mold or spoil.

During the experimental period the squashes were stored in the dry laboratory attic. In order that the squashes should be exposed to a known amount of illumination, they were placed on the floor, 33 inches below a series of four General Electric, 20-watt, daylight tubes 22 inches long. The illumination was maintained 24 hours per day for the experimental period. The squashes were approximately nine inches in diameter and numerous readings with a General Electric light meter showed the illumination to

[1] Contribution No. 629, Massachusetts Agricultural Experiment Station.

be 40 foot-candles at the top of the squashes and 25 foot-candles at the floor beneath them. Obviously, the amount of light to which the experimental squashes were subjected far exceeded the amount of daylight that the squashes would have received had they been stored in a greenhouse during the same period. There were about 460 hours of bright sunshine during the experimental period which was about 40 per cent of the total daylight hours as compared with the 2,448 hours of artificial illumination used for the squashes. The latter was adopted to intensify any possible effect of subjecting squashes to light during winter storage and to provide means for duplicating the illumination for future studies. Comparable squashes were placed in the dark in a space immediately adjoining the illuminated area. The temperature, which was the same for both groups, varied from 5 to 18.9°C.(41 to 66°F.) with an average of 13.9°C.(57°F.) for the four months' experimental period.

Five typical squashes were selected for samples at the beginning of the storage period, and at monthly intervals during the experimental period five squashes from the experimental and the control lots were selected for assay. The seeds, placenta, and hard outer shell were removed and portions from various areas of the squash were promptly cut into small pieces, mixed, and a sufficient quantity was reserved for analytical purposes. The samples of squashes were assayed for water, carotene, reduced ascorbic acid, reducing sugars, and total sugars. The official methods of the Association of Official Agricultural Chemists (1940) were used.

RESULTS

In order to facilitate comparisons of results of the assays of squashes from the different lots, the values included (Table 1) are reported on a dry basis.

TABLE 1

Influence of Light During Storage on Composition of Blue Hubbard Squash

Lot No.	Length of storage	Duration of exposure to light	Weight of squash	Water	Dry basis			
					Carotene	Ascorbic acid	Reducing sugars	Total sugars
	days	*hr.*	*lb.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*
1	0	0	15.0	88.5	21.7	84.9	42.4	61.7
2	28	672	15.0	89.5	23.9	79.7	50.3	64.0
3	28	0	13.1	88.7	28.8	59.8	43.1	57.7
4	60	1,440	15.6	89.1	32.6	53.0	43.9	54.8
5	60	0	12.9	89.6	28.4	63.3	50.1	57.3
6	102	2,448	14.0	90.2	18.7	57.2	52.8	59.2
7	102	0	14.1	88.5	14.9	84.1	41.0	53.8

An attempt was made to select squashes of the experimental and control lots that would be of similar weight. However, it was decided that matching the degree of maturity and other factors was more important than using squashes of exactly equal weights. Hence, the weight of squashes in Lot 3 was less than that of Lot 2 and likewise the weight of Lot 5 was less than that of Lot 4.

The carotene content of squashes, both those exposed to the light and those deprived of light, increased during the first two months of storage and then decreased. The increase in carotene content during storage is in accord with the observations of Snyder and Lachman (1944) who report that each of seven varieties of carrots increased in carotene content during cold storage from November to April. The amount of carotene in the squashes (fresh basis) at the end of storage, i.e., 1.9 and 1.7 mg. per 100 gm., respectively, for Lots 6 and 7, is in agreement with 2.22 mg. per 100 gm. reported by Holmes and Spelman (1946) for Blue Hubbard squash after winter storage. These values for carotene show that Blue Hubbard squash, even after winter storage, contains much more carotene than a large proportion of the fruits and vegetables commonly used for human food.

The amount of reduced ascorbic acid found in the squashes fluctuated both for the squashes stored in the light and in the dark but there was no consistent increase or decrease during the storage period. The range of values for the individual squashes was practically the same as was obtained in an earlier study by Holmes, Smith, and Lachman (1948), but the average amount of reduced ascorbic acid in Blue Hubbard squashes during winter storage was less in the present than in the previous study.

The reducing sugars as well as the total sugar content of the squashes fluctuated during the storage period. However, there was no consistent relation between the results obtained for the sugar content of the squashes that were exposed to light and those that were kept in the dark. Phillips (1946) reported fluctuation in the sugar content of Blue Hubbard squashes during winter storage. The amount of both reducing sugars and total sugars increased during the first three months of storage and then decreased.

It is very evident from the results obtained in this study and those reported in the literature that the composition of individual squashes varies over rather wide limits. Hence, one cannot apply very accurately to squashes purchased at random the average values reported in food tables for the composition of squashes.

The results reported above for the carotene, ascorbic acid, and sugar content of stored Blue Hubbard squashes show that they are a valuable constituent of the human diet even after long winter storage either in the light or in the absence of light.

SUMMARY

Blue Hubbard squashes were grown under known soil, climatic, fertilizer, sunshine, and rainfall conditions. When harvested, the squashes were placed in a greenhouse for two months to eliminate those that would be likely to spoil during the experiment. Then the squashes were divided into two comparable lots and were stored under uniform conditions for 102 days, with the exception that one lot was subjected to a known amount of illumination for 24 hours per day and the second lot was stored in the dark. Assays were made of typical squashes for carotene, reduced ascorbic acid, reducing sugars, and total sugars at the beginning of the experiment and at three subsequent periods. The composition of the individual squashes varied. There was no consistent difference between

the composition of the Blue Hubbard squashes stored in the light and those stored in the dark. The amount of carotene, ascorbic acid, and reducing and total sugars found in the squashes showed that even at the end of long winter storage Blue Hubbard squashes stored either in the light or the absence of light are a valuable source of the constituents of the human diet.

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EFFECT OF DIFFERENT COOKING WATERS ON CALCIUM CONTENT OF CERTAIN VEGETABLES[1,2]

GERALDINE BRYANT[3] AND RUTH JORDAN

Department of Home Economics, Agricultural Experiment Station, Purdue University, Lafayette, Indiana

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The purpose of this study was to compare the effects of hard well water, softened water from the same source, and distilled water on the calcium content of vegetables cooked in the three waters. In the pursuit of this objective other data were collected including loss of weight in the preparation of the vegetables for cooking and change in weight of the vegetables resulting from the cooking process.

A number of studies have been reported on the change in calcium content of vegetables during cooking, a few of which, dealing with the effect of kinds of cooking water, are reviewed here.

Fischer (1931) reported that the extraction of calcium by hard water was less than by soft waters. Horner (1935) found that stringless beans and carrots cooked in distilled water lost about 30 per cent of their calcium but when cooked in hard water, gained a slight amount of calcium. According to Noble and Halliday (1937), all the vegetables they studied, except carrots, contained more calcium when cooked in calcium chloride solution or in Chicago city water (containing 33 to 38 milligrams of calcium per liter) than when raw; the carrots lost less calcium when calcium was present in the cooking medium than when cooked in distilled water. Derstroff (1940) reported that the addition of calcium to calcium-poor water prevented calcium from being extracted from vegetables during cooking. Griebel and Dietze (1942) found no significant influence of the ionic environment, consisting of calcium, sulfate, and sodium ions, on the color, odor, and flavoring materials as well as on the lime content following cooking of vegetables. Ziegelmayer (1944) stated that vegetables cooked in water containing varying concentrations of calcium were found to contain more calcium after cooking than vegetables cooked in distilled water and more than the raw samples. Eheart and Sholes (1945) reported that green beans blanched for 18 minutes in boiling tap water or in tap water to which had been added $NaHSO_4$, or $NaHSO_4$ and Na_2SO_4 contained more calcium than the raw beans. Steam-blanched beans were not significantly different from the raw beans in calcium content. Lee and Whitcombe (1945), using synthetic hard waters for blanching young peas and

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[2] This paper is based upon part of the thesis presented by Geraldine Bryant in partial fulfillment of the requirements for the degree of Master of Science in Home Economics at Purdue University, June, 1947.

[3] Present address of Miss Bryant, Agricultural Experiment Station, University of Hawaii, Honolulu, T. H.

green beans prior to freezing, reported that the calcium content of vegetables blanched in hard water was higher than in the samples in other waters, a result attributed to the large amount of calcium in the blanch water.

EXPERIMENTAL PROCEDURE

Three varieties each of carrots, snap beans, and cabbage were grown and harvested by the Department of Horticulture of Purdue University. The three varieties of cabbage were Early Seneca, Marion Market, and Globe; the varieties of snap beans were Bountiful, Plentiful, and Sure Crop Wax; the varieties of carrots were Nantes, Morse's Bunching, and Chantenay Red Cored. Both younger and more mature carrots were used.

Three kinds of water were used for cooking the vegetables—tap, softened tap, and distilled. The tap water was taken from the Purdue wells. Enough tap water for the experiments was drawn at one time, mixed, and stored in glass bottles. The softened tap water was collected and stored just after the zeolite-type softener had been recharged. The distilled water was taken directly from a still as needed. Analysis showed that tap water contained 92 milligrams of calcium per liter and the softened tap water, 26 milligrams per liter.

The vegetables were weighed, pared as for home use, and reweighed to determine the edible portion. The carrots were quartered lengthwise; the beans were broken into one-inch pieces; the cabbage heads were sliced into small wedges. Representative samples of 50 to 100 grams were weighed. Six of these were cooked, two at a time, in softened tap water, six in tap water, six in distilled water, and the other six were the raw samples. The ones to be cooked were tied in cheesecloth bags. The two samples were cooked each time along with enough more of the vegetable to make four servings, Halliday and Noble (1946). The vegetables were added to 500 milliliters of boiling water in enamelware saucepans with loosely fitting covers. The cabbage was cooked 10 minutes, the younger carrots 20 minutes, and the more mature carrots and snap beans 30 minutes.

The vegetables were drained for five minutes and then weighed. The samples were thoroughly dried and stored in covered glass containers for later analysis. The dried samples were ashed in a muffle furnace and analyzed for calcium by the McCrudden (1911) method with slight modifications.

RESULTS AND DISCUSSION

Data on the waste involved in preparation of the vegetables for cooking (Table 1) show that the greatest waste was in the cabbage. Several outer leaves had to be discarded because of worm holes. The least waste came in snap beans since it was necessary to discard only the tips and a few immature beans. The more mature carrots had less waste than the younger ones. Since the more mature carrots were much larger than the younger ones, the surface area was lower in proportion to the weight; consequently, there was a tendency for the percentage of waste from parings to be lower.

In all cases, the vegetables lost weight in cooking. The percentages of weight lost are shown (Table 2). The cabbage varieties lost the most

TABLE 1

Waste Involved in Preparation of Vegetables for Cooking

Vegetable	Variety	Waste	Average for varieties
		pct.	*pct.*
Cabbage	Early Seneca	28.2	.....
	Marion Market	20.5	.....
	Globe	20.7	23.1
Snap beans	Bountiful	12.0	.....
	Plentiful	12.1	.....
	Sure Crop Wax	13.8	12.6
Carrots[1, 2]	Nantes	19.3	.....
	Chantenay Red Cored	22.0	.....
	Morse's Bunching	18.0	19.8
Carrots[1, 3]	Nantes	16.5	.....
	Chantenay Red Cored	18.2	.....
	Morse's Bunching	15.8	16.8

[1] Carrots were topped before delivery. [2] Harvested in August. [3] Harvested in October

TABLE 2

Weight Lost From Vegetables Owing to Cooking

Vegetable	Average loss			Average for treatments
	Tap water	Distilled water	Softened water	
Cabbage	*pct.*	*pct.*	*pct.*	*pct.*
Early Seneca	14.8	12.3	13.1	13.4
Marion Market	9.7	8.5	9.4	9.2
Globe	12.7	11.5	12.5	12.2
Average for varieties	12.4	10.8	11.7	11.6
Snap beans				
Bountiful	4.2	2.9	3.8	3.6
Plentiful	2.8	2.7	3.2	2.9
Sure Crop Wax	4.4	3.7	4.0	4.0
Average for varieties	3.8	3.1	3.7	3.5
Carrots [1]				
Nantes	8.9	8.2	8.2	8.4
Chantenay Red Cored	8.7	11.0	9.2	9.6
Morse's Bunching	9.3	9.9	8.8	9.3
Average for varieties	9.0	9.7	8.7	9.1
Carrots [2]				
Nantes	10.3	9.6	10.4	10.1
Chantenay Red Cored	12.0	12.1	12.2	12.1
Morse's Bunching	11.1	10.2	10.7	10.7
Average for varieties	11.1	10.6	11.1	11.0
Average for all carrots	.....	.....	.....	10.1

[1] Harvested in August. [2] Harvested in October.

weight, even though they were cooked for the shortest period. The more mature carrots, cooked 10 minutes longer, lost more weight than the younger carrots. The snap beans showed the least change of weight although they were cooked as long as the more mature carrots. There seemed to be no definite trend in percentage of weight lost that could be attributed to the kind of cooking water used.

The average amounts of calcium found in the vegetables are shown (Table 3 and Fig. 1). Of the three varieties of cabbage, in the raw state, the Marion Market and Globe were slightly higher in calcium content than was the Early Seneca. Of the three varieties of uncooked snap beans the Bountiful had a slightly lower calcium content than Plentiful and Sure

TABLE 3

Average Amount of Calcium in Vegetables

Vegetable	Raw	Cooked		
		Tap water[1]	Softened water[2]	Distilled water
	mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Cabbage				
Early Seneca	35.7	36.2[3]	32.5[3]	30.5[3]
Marion Market	42.7	42.3	36.4	35.4
Globe	42.6	40.0	32.9	31.3
Average for all varieties	40.3	39.5	33.9	32.4
Least difference necessary for significance at			5% level	1% level
For varieties			1.54	2.08
For treatments			1.77	2.40
Snap beans				
Bountiful	39.7	41.5	37.9	37.6
Plentiful	42.4	44.5	40.1	39.7
Sure Crop Wax	40.5	42.4	38.0	38.6
Average for all varieties	40.9	42.6	38.7	38.6
Least difference necessary for significance at			5% level	1% level
For varieties			1.16	1.57
For treatments			1.17	1.58
Carrots[4]				
Nantes	31.5	35.7	28.4	29.8
Chantenay Red Cored	33.8	35.1	29.9	30.4
Morse's Bunching	37.2	38.3	32.9	33.6
Average for all varieties	34.2	36.4	30.4	31.3
Least difference necessary for significance at			5% level	1% level
For varieties			1.42	1.83
For treatments			1.64	2.23
Carrots[5]				
Nantes	38.5	38.4	33.6	35.6
Chantenay Red Cored	37.4	37.0	31.8	33.4
Morse's Bunching	39.3	39.1	36.1	35.7
Average for all varieties	38.4	38.2	33.8	34.9
Least difference necessary for significance at			5% level	1% level
For varieties			0.89	1.20
For treatments			1.02	1.39

[1] Contained 92 mg. of calcium per liter of water. [2] Contained 26 mg. of calcium per liter of water. [3] Calculated on the basis of 100 gm. raw vegetable. [4] Harvested in August. [5] Harvested in October.

Crop Wax. In the younger raw carrots the Nantes had a slightly lower calcium content than the other two varieties. In the more mature raw carrots the Chantenay Red Cored was slightly lower in calcium content than Nantes and Morse's Bunching.

Of the three kinds of vegetables in the uncooked state, the beans and cabbage were found to be slightly richer sources of calcium than the carrots. The average amount of calcium for the three varieties of cabbage was 40.3, and for snap beans 40.9 milligrams per 100 grams; whereas for the younger carrots and the more mature carrots the average values were 34.2 and 38.4 milligrams, respectively. From the standpoint of the contribution of calcium made to the diet by a 100-gram portion of vegetable, however, the differences would not seem to be very important.

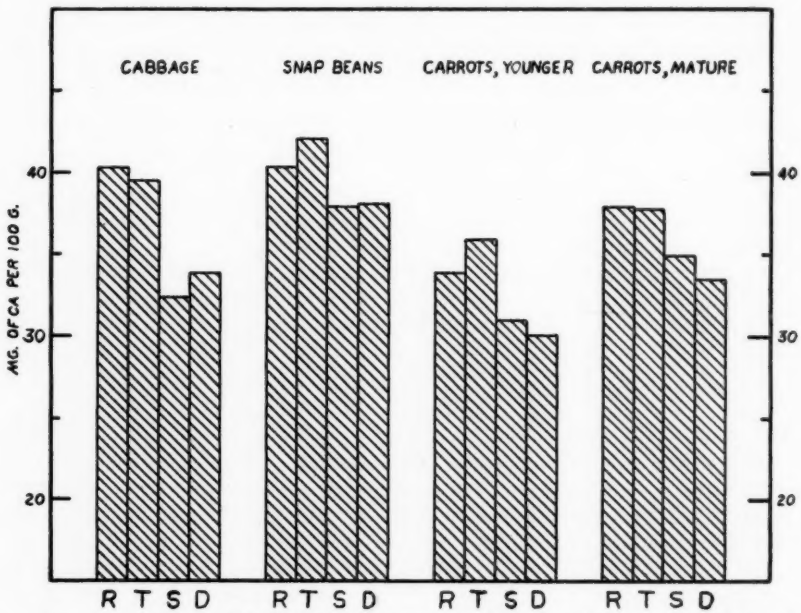

FIG. 1. Average calcium content of three varieties of cabbage, snap beans, and carrots. (R—raw; T—cooked in hard tap water; S—cooked in softened tap water; D—cooked in distilled water.)

The kind of cooking water used was found to affect the amount of calcium retained by the cooked vegetable, as is evident from the data (Table 3). An analysis of variance of the data for each kind of vegetable, made according to the methods of Snedecor (1946), indicated that both treatment and variety affected the calcium content; however, the treatments exerted considerably greater influence than did the varieties. The statistical analysis indicated that in general all varieties tended to be affected in the same way by the various cooking waters.

All the varieties of vegetables cooked in distilled and softened tap water showed a significant loss of calcium content. The cabbage varieties, cooked

only 10 minutes, lost the most calcium. The more mature carrots, cooked 30 minutes, lost on the average more calcium than the younger carrots which were cooked 20 minutes. The snap beans, cooked 30 minutes, lost the least calcium. Since the calcium contents of the vegetables cooked in distilled and softened tap water are so nearly the same, it is assumed that the loss is due to leaching out during the cooking period and not to the excess sodium ions present in the softened tap water.

The Early Seneca cabbage, all varieties of snap beans, and all varieties of the younger carrots showed a gain in calcium when they were cooked in tap water; but only the gains of the Nantes carrots and the bean varieties were significant. In the Marion Market cabbage and all varieties of more mature carrots, the loss was not statistically significant. In the Globe cabbage there was a greater loss of calcium after cooking in tap water, but there was also a correspondingly greater loss when it was cooked in softened tap and distilled water. When tap water containing considerable calcium is used for cooking, it seems that enough calcium is absorbed by the vegetables to offset some of that which might be leached out during the cooking period.

If the softened tap water which was used in this study is typical of softened waters, it is assumed that vegetables cooked in tap water that has been softened will always lose more calcium than the vegetables cooked in the corresponding hard water.

SUMMARY

As a means of studying the effect of certain cooking waters upon the calcium content of vegetables, three vegetables were chosen and analyzed for calcium. The vegetables used were three varieties each of carrots, cabbage, and snap beans.

Data were collected on the amount of waste involved in preparing the vegetables for cooking. Data were also assembled on the change of weight of the vegetables owing to cooking. All the vegetables lost weight, the cabbage varieties losing the most weight and the snap beans, the least.

The analyses showed that the calcium content of vegetables cooked in tap water with 92 milligrams of calcium per liter was about the same, or insignificantly less, or sometimes greater than the calcium content of the raw ones. Vegetables cooked in softened tap water with 26 milligrams of calcium per liter contained less calcium than the raw and sometimes lost as much as vegetables cooked in distilled water. Some factors which apparently affected the amount of calcium retained in cooked vegetables were the amount of calcium present in the cooking water and the length of cooking period.

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COMPARATIVE TENDERNESS AND IDENTIFICATION OF MUSCLES IN WHOLESALE BEEF CUTS

J. M. RAMSBOTTOM AND E. J. STRANDINE
Research Laboratories, Swift & Company, Chicago, Illinois

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Tenderness in beef may be defined as the state of being easily comminuted or masticated. Differences in tenderness occur between carcasses, between cuts within a carcass, between muscles within a cut, and occasionally between parts of the same muscle. For any given grade of beef there is more variation in tenderness than in flavor, texture, aroma, or juiciness. While these general observations are well known, little information has been published on the comparative tenderness of the larger muscles of the beef carcass.

Lehmann and Rumpf (1907) measured the tenderness of a few beef muscles with a mechanical device and reported marked differences in tenderness. Mitchell, Hamilton, and Haines (1928) studied connective tissue as related to tenderness of beef muscle. Most of the data were reported on cuts rather than muscles. Tender muscles were found to be lower in collagen than tough muscles. They reported the elastin to be a small and insignificant portion of the connective tissue in the muscles which were studied.

Paul and McLean (1946) reported on variations between some muscles of the hindquarter of veal. They found large differences between muscles with respect to cooking losses and palatability. These differences usually accounted for a significant portion of the total variation between carcasses of different weights.

While it is generally known that some beef muscles are tender and others are tough, no co-ordinated study has been made to establish a tenderness rating for the muscles of the beef carcass. A project was initiated in these laboratories a few years ago to develop this information as an approach to the study of factors responsible for tenderness. Some of the results were reported by Ramsbottom, Strandine, and Koonz (1945). Additional data are reported in the present paper on the tenderness, composition, weight, and location of 50 representative muscles of the beef side, including those studied in the first paper. These muscles comprise approximately 88 per cent of the lean meat of the beef side. Illustrations are shown of the cut surfaces of the wholesale cuts, identifying the muscles and showing the relationship of the muscles to fat and bone.

EXPERIMENTAL PROCEDURE

The beef selected consisted of three heifer carcasses which averaged 500 pounds dressed weight and graded commercially U. S. Good. The right and left sides of these carcasses were cut into wholesale cuts and the muscles were dissected on the second, third, and fourth days following

slaughter. During this period they were stored at 1.1°C.(34°F.) and a relative humidity of 95 per cent.

The muscles were stripped free of adhering fat, weighed, and samples were taken from the thickest portion of each muscle for histological examination, moisture, fat, and tenderness tests. The samples which were used for tenderness experiments were frozen at —34.4°C.(—30°F.) on the fifth day after slaughter. After one to three weeks' storage at —30°F. the samples were removed and thawed at 2.2°C.(36°F.) in preparation for testing and analysis. Three representative samples were taken from each muscle and composited for determination of moisture, fat, and pH. Steaks were cut from one or more regions of each muscle for tenderness tests on the cooked meat.

Difficulties in obtaining uniform cooking with small muscles by standard cooking methods are obvious. Therefore, after preliminary experiments it was decided to cook the muscles in lard which was kept at a constant temperature of 121.1°C.(250°F.). Thermometers were inserted in the meat and each piece was removed from the lard upon reaching an internal temperature of 76.7°C.(170°F.); however, the temperature of the meat continued to rise one or two degrees after removal. From six to 15 minutes were required to cook the meat under these conditions. The length of cooking time depended not only on weight but also on thickness of the meat.

The Warner-Bratzler shear (1928) was used to obtain shear readings on both the raw and cooked meat; four or more readings were made at right angles to the grain on each piece of raw or cooked meat. The results were expressed in the number of pounds of force required to shear a sample one-half inch in diameter. Therefore, increase in shear values indicated decrease in tenderness. The temperature of the raw and cooked meat when sheared was 2.2°C.(36°F.) and 23.9 to 29.4°C.(75 to 85°F.), respectively. Each sample of cooked meat was evaluated organoleptically for tenderness as (1) very tough, (2) tough, (3) slightly tough, (4) medium, (5) slightly tender, (6) tender, or (7) very tender.

Representative samples of both raw and cooked muscles were prepared for histological examination. Small pieces of the raw muscles were fixed in 10 per cent formalin, dehydrated in alcohol, embedded in paraffin, and sectioned. Weigert's elastic connective tissue stain was used to show the distribution of the elastic connective tissue, while Mallory's triple connective tissue stain was used to demonstrate the collagenous connective tissue. From the cooked muscles larger samples of muscles (approximately 1½ x ¾ inches) were sectioned on the freezing microtome and stained with gentian violet and Sudan III.

Muscles were compared as to the relative amounts of elastic connective tissue and collagenous tissue. The following arbitrary numerical evaluations were used in making the comparison:

Relative amount of tissue present	Elastic fibers	Collagenous fibers
None	1	1
Small	3	3
Medium	5	5
Large	7	7

By adding the numerical evaluation for the elastic fibers and the collagenous fibers, a figure was obtained for each muscle which represented the histological tenderness rating. For example, a muscle with large numbers of elastic and collagenous fibers would rate 14, whereas one with small amounts of both types of fibers would rate only 6. These histological scores were compared with shear readings and with organoleptic evaluations for the purpose of developing information on the tenderness of the beef.

EXPERIMENTAL RESULTS

Variations in Fat, Moisture, and pH: The muscles varied widely in fat and moisture as shown (Table 1). The intercostal muscles located between the ribs had an average fat content of 18.1 per cent and a moisture content of 62.5 per cent, whereas at the other extreme the extensor carpi radialis of the foreshank had a fat content of 1.5 per cent and a moisture content of 76 per cent. These figures represent the high and low values for fat and moisture. The average fat and moisture contents of all muscles were 5.7 and 72.2 per cent, respectively. The pH values ranged from 5.5 to 6 with an average pH of 5.7.

Weight of Muscles and Proportion of Muscles in Different Wholesale Cuts: The average weight of each muscle is recorded (Table 1) together with the proportions of the different muscles located in the following wholesale cuts: (1) round, (2) rump, (3) sirloin, (4) short loin, (5) flank, (6) rib, (7) navel, (8) chuck, (9) brisket, and (10) foreshank. Some muscles, for instance the peroneus tertius and gastrocnemius, are located entirely in the round, whereas other muscles, such as the external oblique, extend over a large part of the side and consequently portions are located in six different wholesale cuts.

Tenderness Indices of Beef Muscles: Tenderness data on beef muscles as indicated by the shear of the cooked beef, shear of the raw beef, tenderness rating, and collagenous and elastic fiber rating are reported (Table 2). The shear of the cooked beef was used as a standard, inasmuch as it is an objective test and experience has indicated that it is highly correlated with tenderness of beef as rated by a tenderness committee.

As stated in the experimental procedure, numerical values were given to the tenderness ratings and to the collagenous and elastic fiber ratings. The values for collagenous and elastic fibers were combined to obtain a histological rating. These values and the shear values (Table 2) on raw and cooked beef were used in determining correlation coefficients as follows:

Shear cooked beef: tenderness rating	0.9
Shear cooked beef: shear raw beef	0.6
Shear cooked beef: histological rating	0.6

All of these coefficients are significant according to Snedecor (1940), the coefficient between the shear of the cooked beef and the tenderness rating being highly significant.

A comparison of the shear values on raw and cooked beef shows that shear values for the cooked beef were greater for 35 of the 50 muscles. This indicates that most muscles become somewhat less tender when heated quickly to 170°F. Shear values for the cooked muscles ranged from a low of 7.1 pounds, required to shear circular, one-half-inch samples of the

TABLE 1

Analyses and Weights of Beef Muscles and Percentages of Muscles in Wholesale Cuts

Muscles (listed from posterior to anterior end)	Moisture	Fat	pH	Average weight	Muscle weight in different cuts[1]				
	pct.	*pct.*		*lb.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
1. Peroneus tertius	74.4	2.3	5.8	0.8	(1) 100	.....	.....	.....	.....
2. Gastrocnemius	74.0	2.0	5.6	2.6	(1) 100	.....	.....	.....	.....
3. Superficial digital flexor	72.2	4.1	5.7	0.5	(1) 100	.....	.....	.....	.....
4. Vastus intermedius	72.6	7.8	5.6	1.0	(1) 100	.....	.....	.....	.....
5. Vastus medialis	75.0	2.8	5.6	1.2	(1) 83	(3) 17	.....	.....	.....
6. Vastus lateralis	73.9	2.9	5.5	3.3	(1) 85	(3) 15	.....	.....	.....
7. Rectus femoris	74.4	2.9	5.5	2.8	(1) 68	(3) 32	.....	.....	.....
8. Sartorius	73.2	3.8	5.6	0.4	(1) 80	(3) 20	.....	.....	.....
9. Pectineus	73.8	2.9	5.5	0.9	(1) 100	(2)[2]	.....	.....	.....
10. Gracilis	74.0	3.8	5.5	1.8	(1) 100	(2)[2]	.....	.....	.....
11. Adductor	74.1	2.5	5.6	1.8	(1) 100	(2)[2]	.....	.....	.....
12. Semimembranosus	74.2	2.9	5.4	8.7	(1) 100	(2)[2]	.....	.....	.....
13. Semitendinosus	73.4	2.6	5.5	3.2	(1) 100	(2)[2]	.....	.....	.....
14. Biceps femoris	72.4	5.1	5.6	10.2	(1) 74	(2) 16	(3) 10	.....	.....
15. Tensor fascia latae	72.9	5.3	5.6	2.0	(1) 10	(3) 90	.....	.....	.....
16. Obturator externus	71.3	6.5	5.8	0.7	(1) 70	(2) 30	.....	.....	.....
17. Iliacus	73.9	4.8	5.7	1.1	(1)[2]	(3) 100	.....	.....	.....
18. Gluteus medius	72.2	5.3	5.6	4.4	(2) 11	(3) 89	(4)[2]	.....	.....
19. Psoas major	70.4	8.2	5.8	2.6	(1)[2]	(3) 38	(4) 62	.....	.....
20. Psoas minor	67.0	11.6	5.7	0.5	(3) 40	(4) 60	.....	.....	.....
21. Multifidis dorsi	70.0	6.7	5.8	3.0	(2)[2]	(3) 13	(4) 30	(6) 57	.....
22. Longissimus dorsi	72.9	6.3	5.7	9.6	(3) 5	(4) 51	(6) 40	(8) 4	.....
23. Longissimus costarum	69.6	9.6	6.0	0.6	(4) 16	(6) 68	(8) 16	.....	.....
24. Internal oblique	72.9	6.3	5.7	3.0	(3) 47	(4) 33	(5) 20	.....	.....

TABLE 1 *(Concluded)*

25. External oblique	67.4	11.5	5.7	2.8	(4) 29	(5) 3	(6)[2]	(7) 68	(9)[2]
26. Rectus abdominis	70.0	8.8	5.6	3.6	(4) 44	(5) 17	(7) 39	.....	.....
27. Cutaneous	67.9	10.1	5.8	2.7	(5) 48	(6) 4	(7) 37	(8) 11	.....
28. Diaphragm	67.9	10.5	5.9	1.2	(4)[2]	(6) 25	(7) 75	.....	.....
29. Intercostals	62.5	18.1	6.0	4.1	(4)[2]	(6) 51	(7) 24	(8) 15	(9) 10
30. Latissimus dorsi	72.5	5.0	5.5	3.0	(6) 66	(8) 34	.....	.....	.....
31. Spinalis	67.8	12.1	5.7	1.0	(6)[2]	(8) 100	.....	.....	.....
32. Rhomboideus	73.0	4.1	5.5	1.6	(6) 19	(8) 81	.....	.....	.....
33. Trapezius	73.0	4.8	5.7	2.0	(6) 35	(8) 65	.....	.....	.....
34. Subscapularis	72.8	4.7	5.7	1.6	(6) 6	(8) 94	.....	.....	.....
35. Complexus	74.2	3.6	5.8	1.0	(6)[2]	(8) 100	.....	.....	.....
36. Infraspinatus	72.7	6.0	5.7	3.0	(6)[2]	(8) 100	.....	.....	.....
37. Serratus ventralis	72.9	5.8	5.7	6.2	(6) 16	(7) 13	(8) 69	(9) 2	.....
38. Transversus abdominis	69.6	8.9	5.7	1.6	(7) 100	.....	.....	.....	.....
39. Deep pectoral	72.7	4.3	5.5	4.3	(7)[2]	(8) 40	(9) 60	.....	.....
40. Superficial pectoral	73.3	5.7	5.7	2.6	(7) 27	(8)[2]	(9) 63	(10)[2]	.....
41. Supraspinatus	72.3	6.1	5.7	2.1	(8) 100	.....	.....	.....	.....
42. Teres major	73.6	4.6	5.7	0.6	(8) 100	.....	.....	.....	.....
43. Deltoid	71.4	2.8	5.6	0.6	(8) 100	.....	.....	.....	.....
44. Splenius	74.5	2.6	5.7	0.8	(8) 100	.....	.....	.....	.....
45. Brachiocephalicus	74.2	5.7	5.7	1.8	(8) 100	(10)[2]	.....	.....	.....
46. Sternocephalicus	71.3	7.4	6.0	0.7	(8) 100	.....	.....	.....	.....
47. Brachialis	74.7	3.4	5.8	0.6	(8) 100	.....	.....	.....	.....
48. Triceps brachii	72.7	5.2	5.7	5.2	(8) 80	(10) 20	.....	.....	.....
49. Biceps brachii	74.6	3.1	5.8	0.8	(8) 75	(10) 25	.....	.....	.....
50. Extensor carpi radialis	76.0	1.5	5.7	1.0	(8)[2]	(10) 100	.....	.....	.....
Average	72.2	5.7	5.7	2.5	.....	.....	.....	.....	.....

[1] Wholesale cuts are identified as follows: (1) round, (2) rump, (3) sirloin, (4) short loin, (5) flank, (6) rib, (7) navel, (8) chuck, (9) brisket, (10) foreshank. [2] Very small portion in wholesale cut; weight not recorded.

TABLE 2

Tenderness Indices of Beef Muscles

Muscles (listed from posterior to anterior end)	Shear (cooked beef)	Shear (raw beef)	Tenderness rating (cooked beef)	Collagenous fiber rating (raw beef)[1]	Elastic fiber rating (raw beef)[1]
	lb.	*lb.*			
1. Peroneus tertius	10.8	12.0	Tough	M	M
2. Gastrocnemius	9.5	8.4	Medium	M	S
3. Superficial digital flexor	10.0	13.3	Tough	L	S
4. Vastus intermedius	9.2	6.9	Sl. tough	M	S
5. Vastus medialis	9.9	6.3	Medium	M	S
6. Vastus lateralis	11.3	5.8	Sl. tough	M	M—
7. Rectus femoris	9.4	6.8	Sl. tough	M	S
8. Sartorius	10.3	9.0	Medium	M	M
9. Pectineus	8.1	5.0	Medium	M	S
10. Gracilis	11.5	9.8	Sl. tough	M	S
11. Adductor	10.6	5.2	Tough	M	S
12. Semimembranosus	11.9	6.3	Sl. tough	M	S
13. Semitendinosus	11.1	10.9	Sl. tough	L	L
14. Biceps femoris	9.1	7.4	Medium	M	M
15. Tensor fascia latae	10.8	6.7	Medium	M	S
16. Obturator externus	7.2	8.6	Sl. tender	M	S
17. Iliacus	7.5	5.0	Tender	M	S
18. Gluteus medius	8.2	4.7	Sl. tender	M	S
19. Psoas major	7.1	4.0	Very tender	S	S
20. Psoas minor	7.1	4.9	Tender	M	S
21. Multifidis dorsi	8.0	5.3	Sl. tender	M	S
22. Longissimus dorsi	8.3	3.8	Sl. tender	M	S
23. Longissimus costarum	7.9	10.0	Sl. tender	L	S
24. Internal oblique	12.6	4.7	Very tough	M	M—
25. External oblique	11.9	13.4	Tough	L	M
26. Rectus abdominis	11.6	8.1	Sl. tough	M	S
27. Cutaneous	13.8	26.0	Very tough	L	L
28. Diaphragm	10.0	8.3	Sl. tough	M	S
29. Intercostals	10.0	8.3	Sl. tough	M	M
30. Latissimus dorsi	12.5	11.2	Very tough	L	L
31. Spinalis	8.4	6.3	Medium	M	M
32. Rhomboideus	16.3	8.6	Very tough	M	L
33. Trapezius	14.0	17.4	Very tough	L	L
34. Subscapularis	9.9	5.8	Sl. tough	M	S
35. Complexus	14.4	12.4	Very tough	L	M+
36. Infraspinatus	8.0	5.7	Sl. tender	M	S
37. Serratus ventralis	9.3	7.5	Medium	M	S+
38. Transversus abdominis	13.1	8.8	Very tough	L—	M
39. Deep pectoral	12.3	12.5	Very tough	L	M
40. Superficial pectoral	15.3	10.4	Very tough	L	M
41. Supraspinatus	9.2	9.5	Medium	M	S
42. Teres major	11.1	9.4	Sl. tough	M	M
43. Deltoid	11.9	17.0	Tough	L	M
44. Splenius	13.4	8.8	Very tough	L	M
45. Brachiocephalicus	13.6	18.0	Very tough	L	M
46. Sternocephalicus	15.6	19.8	Very tough	L	S
47. Brachialis	11.9	16.3	Tough	M	M
48. Triceps brachii	8.5	7.8	Medium	M	S
49. Biceps brachii	8.8	12.8	Sl. tough	M+	M
50. Extensor carpi radialis	11.6	13.9	Tough	L	S

[1] Collagenous and elastic fiber rating: S=small, M=medium, L=large.

psoas major (tenderloin), to a high of 16.3 pounds for the rhomboideus, a rather small, very tough muscle located in the shoulder or chuck. These results on tenderness are in agreement with data reported in our first paper on this subject, Ramsbottom, Strandine, and Koonz (1945).

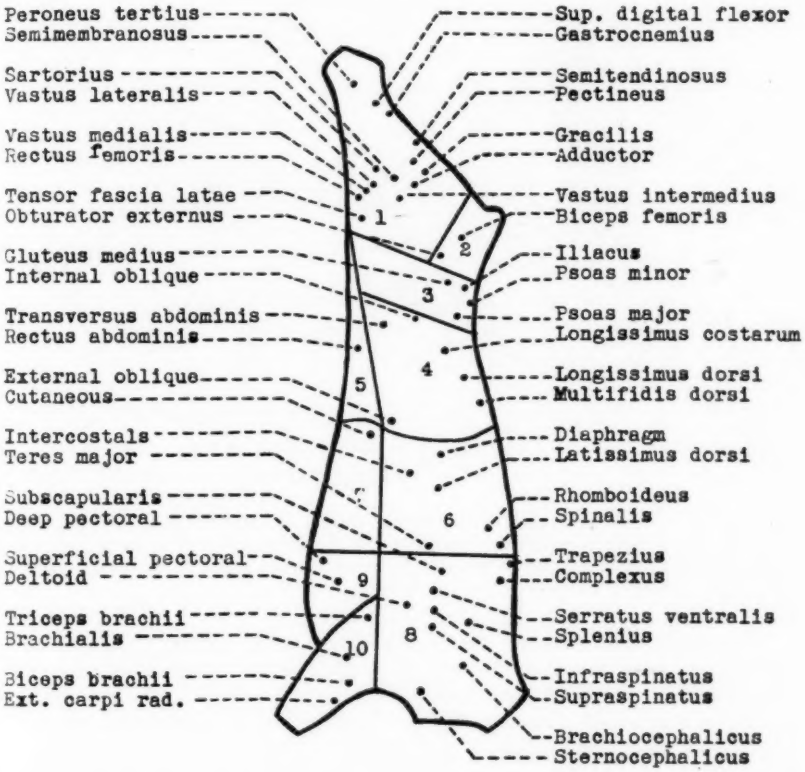

FIG. 1. Chart showing approximate location of muscles studied and identification of wholesale cuts: (1) round, (2) rump, (3) sirloin, (4) short loin, (5) flank, (6) rib, (7) navel, (8) chuck, (9) brisket, (10) foreshank.

Identification of Muscles in Wholesale Cuts: In commercial handling, a side of beef is cut into primal cuts as shown (Fig. 1). The first step in this operation is to cut the half-beef carcass or beef side into forequarter and hindquarter. This cut may be made so as to leave none, one, or more ribs on the hindquarter depending on local custom. In this investigation the forequarter and hindquarter were divided between the 12th and 13th ribs, thereby leaving the 13th rib in the hindquarter. For purposes of the present study, the side of beef was divided into the wholesale cuts listed (Fig. 1).

Illustrations showing the cut surface of wholesale cuts of beef are helpful in identifying the muscles. Therefore, drawings were made of the

cut surfaces of all wholesale cuts together with a cross section of the round. The muscles are identified and the shear values of the cooked beef are given for each muscle at the location of the cross section (Figs. 2 to 13). These shear values do not necessarily check with the shear values reported (Table 2) because the latter figures are averages made of values in different parts of the muscle.

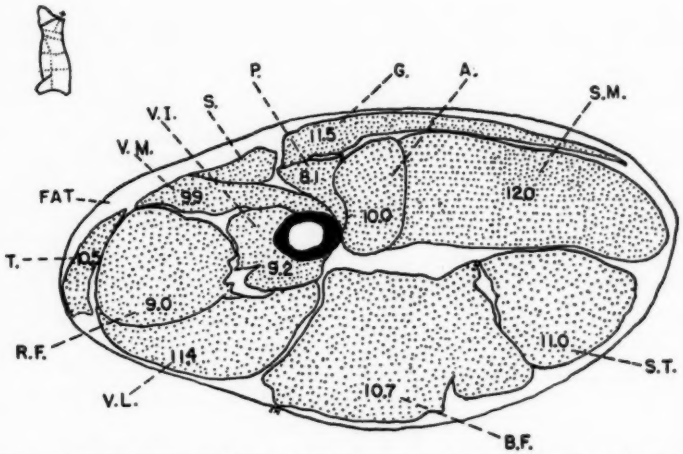

FIG. 2. Average shear readings of cooked muscles in round of beef: A., adductor; B.F., biceps femoris; G. gracilis; P., pectineus; R.F., rectus femoris; S., sartorius; S.M., semimembranosus; S.T., semitendinosus; T., tensor fascia latae; V.I., vastus intermedius; V.L., vastus lateralis; V.M., vastus medialis.

A cross section through the center of the round (Fig. 2) is the only illustration that does not show a cut surface between two wholesale cuts. The solid line in the smaller drawing gives the location of the cut surface shown in the larger drawing. Twelve muscles are identified and the relative tenderness of the muscle is indicated by the shear values for the cooked beef which appear in the illustration. The pectineus, a relatively small muscle, was the most tender and the semimembranosus was indicated to be the least tender muscle.

Most of the muscles of the round of good-quality beef are suitable for steaks. Exceptions include the superficial digital flexor, peroneus tertius, and other smaller muscles in the lower part of the round which are high in connective tissues. These latter muscles are often sliced and used for stews or ground and used in hamburger.

The cut surface between round and rump (Fig. 3) shows that the biceps femoris makes up most of the lean meat of the rump. There is considerable variation in the tenderness of this muscle. Ramsbottom, Strandine, and Koonz (1945) reported that the biceps femoris was progressively more tender from the posterior portion located in the round to the anterior portion located in the sirloin. The rump may be trimmed and used as a roast or it may be cut into steaks.

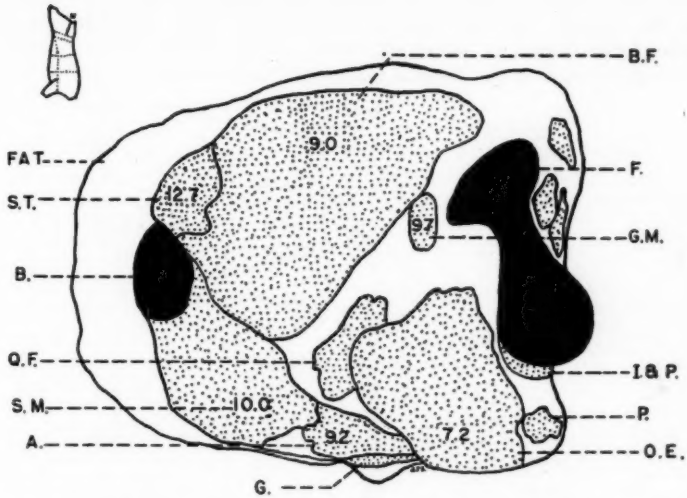

FIG. 3. Average shear readings of cooked muscles in rump of beef: A., adductor; B., bone; B.F., biceps femoris; F., femur; G., gracilis; G.M., gluteus medius; I. and P., iliacus and psoas major; P., pectineus; O.E., obturator externus; Q.F., quadratus femoris; S.M., semimembranosus; S.T., semitendinosus.

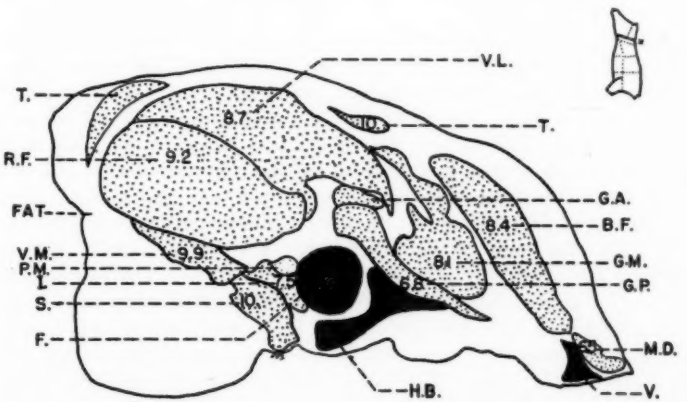

FIG. 4. Average shear readings of cooked muscles in full loin of beef. B.F., biceps femoris; F., femur; G.A., gluteus accessorius; G.M., gluteus medius; G.P., gluteus profundus; H.B., hip bone; I. iliacus; M.D., multifidis dorsi; P.M., psoas major; R.F., rectus femoris; S., sartorius; T., tensor fascia latae; V., vertebra; V.L., vastus lateralis; V.M., vastus medialis.

The cut surface between the round and sirloin (Fig. 4) indicates that all of the muscles are somewhat more tender than those in the center portion of the round as indicated by the shear values of the cooked beef. The rectus femoris is the largest muscle at this location.

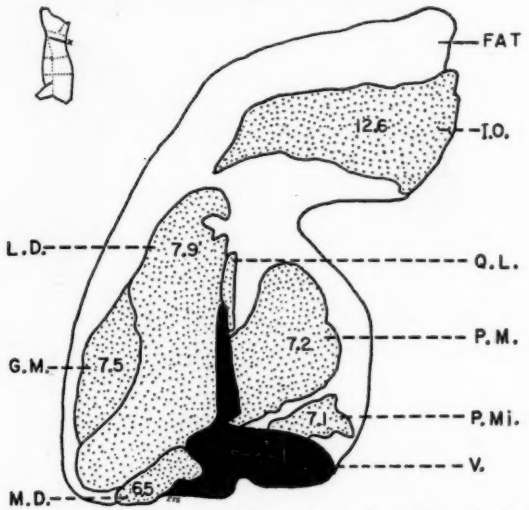

FIG. 5. Average shear readings of cooked muscles in short loin of beef: G.M., gluteus medius; I.O., internal oblique; L.D., longissimus dorsi; M.D., multifidis dorsi; P.M., psoas major; P.Mi., psoas minor; Q.L., quadratus lumborum; V. vertebra.

Except for the internal oblique, which is shown in the lateral part of the cut surface between the sirloin or loin end and short loin (Fig. 5), these muscles average higher in tenderness than those of any other wholesale cut. Two muscles are prominent at this location—longissimus dorsi and psoas major. The sirloin and short loin are trimmed and used for steaks. The psoas major, also known as the fillet or tenderloin, may be removed from the short loin and sirloin and cut into tenderloin steaks. In addition, the longissimus dorsi ("eye muscle"), with adjoining muscles and cover fat, may be stripped from the lumbar vertebrae and bone appendages and then cut into boneless strip steaks. The cut surfaces of the flank are shown (Figs. 6 and 7). As illustrated, there are a number of thin muscles in this cut and much fatty and connective tissue. The most important muscle in the beef flank is the rectus abdominis, part of which is the flank steak. The remainder of the cut is used in making hamburger and processed meats.

A cross section between the 12th and 13th ribs (Fig. 8) shows the posterior cut surface of the wholesale rib. The longissimus dorsi makes up the largest part of the lean meat at this location. The wholesale rib cut is generally trimmed and cut into steaks or standing rib roasts or it may be boned and rolled, then cut into boneless rolled roasts.

The plate, which is made up of the brisket and navel end, is characterized by thin, flat muscles separated by layers of fatty and connective

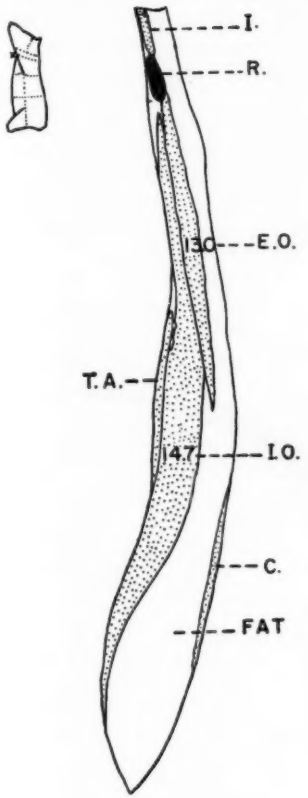

FIG. 6

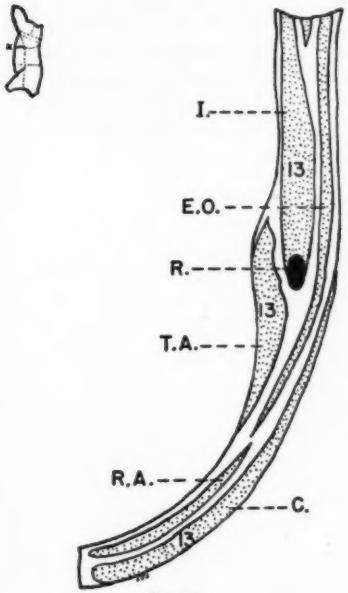

FIG. 7

FIG. 6. Average shear readings of cooked muscles in beef flank: C., cutaneous; E.O., external oblique; I., intercostal; I.O., internal oblique; R., rib; T.A., transversus abdominis.

FIG. 7. Average shear readings of cooked muscles in beef flank (anterior cut surface): C., cutaneous; E.O., external oblique; I., intercostal; R., rib cartilage; R.A., rectus abdominis; T.A., transversus abdominis.

tissues. This is illustrated (Figs. 9 and 11) in cross sections between the rib and navel end and between the brisket and navel end, respectively. The thin muscles in this section of the carcass are not suitable for roasts and steaks because of lack of tenderness and because of layers of fatty connective tissues between muscles. The brisket is often cured and sold as corned beef. Short ribs are cut from the part of the navel end adjoining the rib. The remainder of the navel end is used for stew beef, ground beef, and as raw material for processed meats.

The cut surface between the fifth and sixth ribs (Fig. 10), which divides the wholesale rib from the chuck, shows that there are many more muscles of variable tenderness at this point than in the loin; as a result, cuts from the chuck are cooked by different methods—roasting, pot roasting, frying, and stewing—depending upon the grade of meat and section involved.

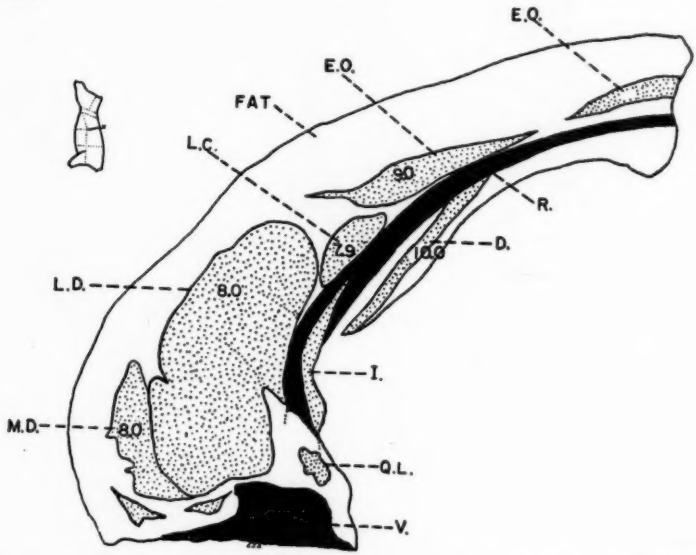

FIG. 8. Average shear readings of cooked muscles in rib of beef: D., diaphragm; E.O., external oblique; I., intercostal; L.C., longissimus costarum; L.D., longissimus dorsi; M.D., multifidis dorsi; Q.L., quadratus lumborum; R., rib; V., vertebra.

The cross section which divides the chuck from the foreshank and brisket is illustrated (Fig. 12). The muscles vary widely in tenderness at this location from the fairly tender triceps brachii to the very tough superficial pectoral.

A number of important muscles are not shown in the two illustrations of the chuck, including two fairly tender muscles—the supraspinatus or so-called "Scotch tender" and the infraspinatus—which are located on the scapula and separated by the spine of the scapula. In addition, tough muscles in the neck region, including the sternocephalicus, are not shown in these illustrations. The muscles of this latter group are generally used

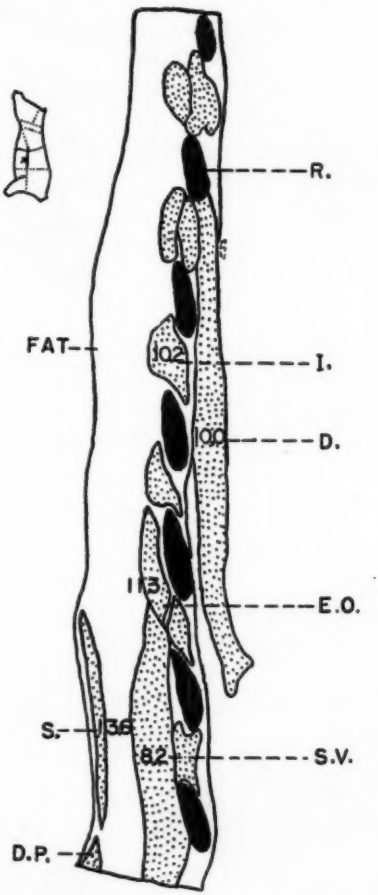

FIG. 9. Average shear readings of cooked muscles in beef plate: D., diaphragm; D.P., deep pectoral; E.O., external oblique; I., intercostal; R., rib; S., skin or integumentary muscle; S.V., serratus ventralis.

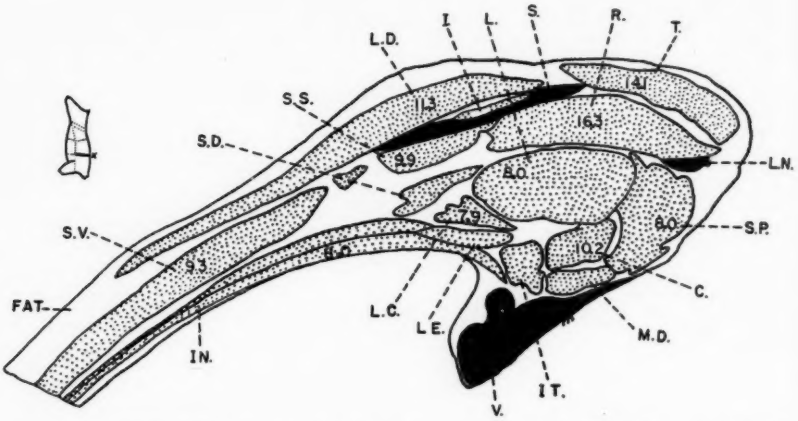

FIG. 10. Average shear readings of cooked muscles in chuck of beef (posterior end): C., complexus; I., infraspinatus; IN., intercostal; IT., intertransversales; L., longissimus dorsi; L.C., longissimus costarum; L.D., latissimus dorsi; L.E., levator costarum; L.N., ligamentum nuchae; M.D., multifidis dorsi; R., rhomboideus; S., scapula; S.D., serratus dorsalis; S.P., spinalis; S.S., subscapularis; S.V., serratus ventralis; T., trapezius; V., vertebra.

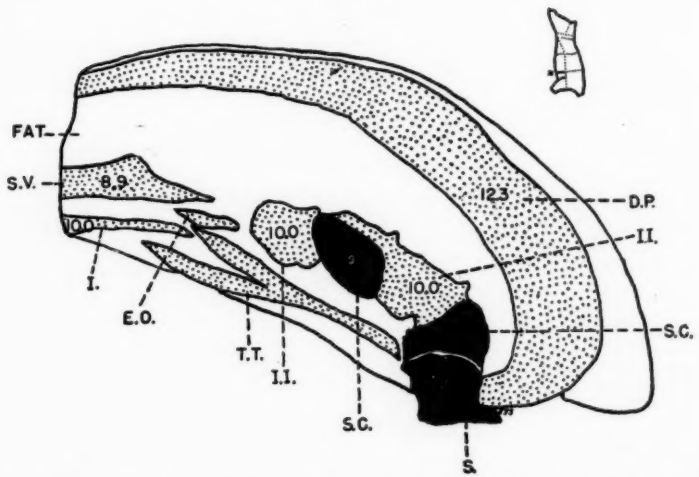

FIG. 11. Average shear readings of cooked muscles in brisket of beef: D.P., deep pectoral; E.O., external oblique; I., intercostal; I.I., internal intercostals; S., sternum; S.C., sternal cartilages; S.V., serratus ventralis; T.T., transversus thoracis.

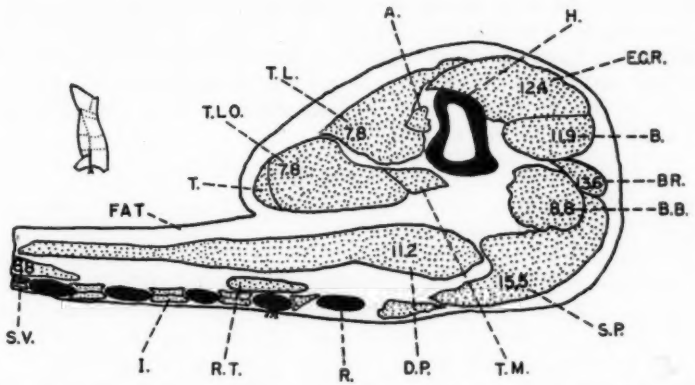

FIG. 12. Average shear readings of cooked muscles in chuck of beef (shank end): A., anconeus; B., brachialis; B.B., biceps brachii; B.R., brachiocephalicus; D.P., deep pectoral; E.C.R., extensor carpi radialis; H., humerus; I., intercostals; R., ribs; R.T., rectus thoracis; S.P., superficial pectoral; S.V., serratus ventralis; T., tensor fascia antibrachii; T.L., triceps brachii, lateral head; T.L.O., triceps brachii, long head; T.M., triceps brachii, medial head.

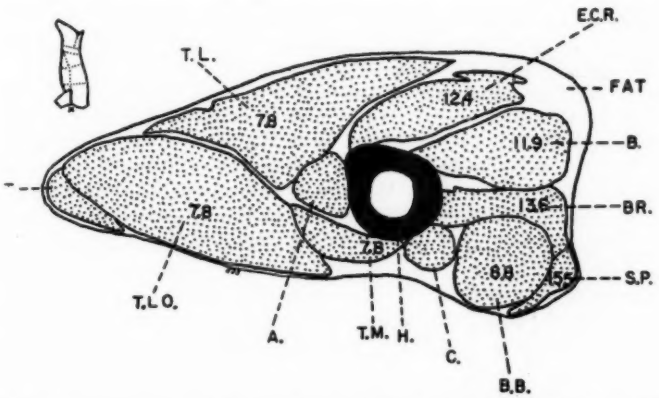

FIG. 13. Average shear readings of cooked muscles in shank of beef: A., anconeus; B., brachialis; B.B., biceps brachii; B.R., brachiocephalicus; C., coracobrachialis; E.C.R., extensor carpi radialis; H., humerus; S.P., superficial pectoral; T. tensor fascia antibrachii; T.L., triceps brachii, lateral head; T.L.O., triceps brachii, long head; T.M., triceps brachii, medial head.

for pot roasts, stews, and ground beef. The more tender portions of the chuck may be used for steaks, dry roasts, and pot roasts.

The long head and lateral head of the triceps brachii extend into the foreshank cut from the chuck (Fig. 13); however, this fairly tender muscle represents only 16 per cent of the lean meat in the foreshank. Most of the muscles in the shank are rather tough; shank meat is generally used as stew meat or ground for hamburger.

SUMMARY

Fifty of the larger muscles were dissected from the wholesale cuts of three U. S. Good beef carcasses. They were weighed, and their percentage of the total weight in each wholesale cut was recorded. Moisture, fat, and pH determinations were made. Tenderness was determined by committee rating, histological rating, and a shearing device. Illustrations were prepared of cut surfaces of the wholesale cuts showing the relationships of the muscles to each other and to adhering fatty tissue and bone.

The beef muscles varied greatly in weight, moisture and fat content, pH, and tenderness. It is apparent from these data that the commercial practice of grouping muscles of similar tenderness should be extended, so that the occurrence of both tough and tender muscles within a roast or steak would be eliminated in so far as it is practical.

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PRESERVATION OF INTACT EGGS BY SEALING WITH CHEMICAL AGENTS

ALEXIS L. ROMANOFF AND W. D. YUSHOK
Agricultural Experiment Station, Cornell University, Ithaca, New York

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A newly laid hen's egg is in equilibrium with an atmosphere having a relative humidity of 99.5 per cent, Sharp and Stewart (1936), and containing 10 per cent carbon dioxide at 25°C.(77°F.), Sharp (1937). If an egg is kept in the air at room temperature or even under ordinary cold-storage conditions, losses of moisture and carbon dioxide from the egg through the porous shell are to be expected. Accompanying these losses are the various physicochemical changes which contribute to the poor appearance of a stale egg.

The most feasible method of preventing the escape of carbon dioxide and water is that of sealing the pores of the shell by means of a coating or an impregnating agent. As early as 1807 eggs were preserved in Holland by dipping in linseed oil, Spamer (1931). An extensive bibliography on chemical agents used prior to 1920 was presented by Jones and DuBois (1920). As a result of their own experiments they concluded that aluminum soap dissolved in gasoline is a good sealing agent for eggs. Almy, Macomber, and Hepburn (1922) demonstrated that dipping eggs in aqueous solutions of various acids, inorganic salts, or soap failed to reduce appreciably losses in weight of eggs during subsequent storage. According to these workers, the only aqueous solution that produced a significant sealing effect was that of five per cent sulfuric acid. The value of mineral oils in decreasing the permeability of the shell varied according to their viscosities, the more viscous oil having a greater sealing effect.

Swenson and Mottern (1930) noted that under the same holding conditions unoiled eggs lost 13 per cent of their total weight and those dipped in oil at atmospheric pressure lost two per cent of their weight. The moisture lost from oil-dipped eggs was 15 per cent of that from untreated eggs. In another experiment untreated and oil-dipped eggs lost 7.71 and 1.60 per cent in weight, respectively, Swenson and James (1931). In this case the loss in weight of the oil-coated eggs was 21 per cent of the loss from the controls. When the eggs were dipped in oil under vacuum, the effectiveness of the oil treatment was increased considerably. The method employing vacuum, however, involves the use of expensive and elaborate equipment.

Rosser, White, Woodcock, and Fletcher (1942) did a considerable amount of work on preserving eggs under the adverse storing and shipping conditions experienced during the war. They concluded that none of the sealing agents (oil, vaseline, or polyvinyl alcohol), when used alone, sealed the egg perfectly.

The objective of this study is to compare the effectiveness of various substances as sealing agents of intact eggs. In surveying these chemical

agents two guiding tests were selected: (1) the change in weight of the eggs during storage, which depends almost entirely upon the permeability of the sealing agent to water vapor, and (2) the change in pH of the albumen, which gives some indication of the escape of carbon dioxide through the coated shell.

EXPERIMENTAL PROCEDURE

Fresh, infertile eggs of uniform shell texture from White Leghorn hens were candled to remove cracked and otherwise defective eggs. The selected eggs were immersed at room temperature in each aqueous solution of the acids, bases, and salts for a one-minute period. When the eggs were dipped in solutions kept at 100°C.(212°F.), or in mineral-oil mixtures at room temperature, the immersion time was decreased to four seconds.

After treatment the eggs were allowed to stand for a 15-minute period to permit draining and drying of the liquid. Each egg was then weighed to the nearest 0.1 gram and was stored in a forced-draft incubator regulated at 37.5°C.(99.5°F.) and 60 per cent relative humidity. After a 10-day storage period the weight of each egg was taken, and the pH of a five-ml. sample of its dense albumen was measured by means of a Beckman laboratory model pH meter using a glass electrode.

The relative permeability of a sealing agent to water vapor under the conditions of the test was calculated by dividing the percentage of weight lost from the untreated eggs into the percentage weight lost by the treated eggs. This figure was then subtracted from 100 to give the relative impermeability.

RESULTS

Lactic Acid as a Sealing Agent: The data (Table 1) indicate that among the acids tested, 1.0 normal lactic acid was most effective in decreasing the loss in weight of eggs and in slowing down the increase in pH of the albumen. Dipping eggs in aqueous solutions of acidified calcium lactate or sodium lactate decreased somewhat the permeability of their shells to moisture.

An attempt was made to deposit a silica-gel coating on the shell by dipping the egg in a solution made by adding sodium silicate to lactic acid until the pH of the mixture was 3. More favorable results were obtained when the silicate-lactate solution was used in conjunction with the flash-heat treatment, Romanoff and Romanoff (1944). That the major sealing ability is due to the lactate and not to the silica gel is suggested by the low relative impermeability of 11 per cent obtained when the sodium silicate was neutralized with hydrochloric acid instead of lactic acid.

Alkaline Solutions in Quick-Dip Process: Numerous experiments have shown that sodium silicate (water glass) solution is a good preservative when eggs are continuously immersed in it, Jones and DuBois (1920), Hall (1945), and Romanoff (1948). When eggs were dipped in sodium silicate solutions for a one-minute period, the loss in weight was not noticeably different from that of untreated eggs. Similar results were obtained with saturated calcium hydroxide solution.

Treatment of eggs with sodium hydroxide, with sodium tetraborate, or with calcium hypochlorite did not change appreciably their permeability

TABLE 1

Effect of Various Chemical Agents Upon Relative Impermeability of Egg Shells[1] to Water Vapor and Upon pH of Albumen[2]

Treatment	Relative impermeability of shell	Alkalinity of albumen[3]
	pct.	*pH*
Mineral-oil mixtures		
Lactic acid, 2 per cent, and stearic acid, 1 per cent	99	7.7
Lactic acid, 0.5 per cent, and stearic acid, 1 per cent	98	7.8
Stearic acid, 1 per cent	95	7.7
Acetic acid, 0.5 per cent	95	7.8
Mineral oil		
High viscosity—3.14 centipoises[4]	88	7.8
Low viscosity—1.16 centipoises[4]	86	8.0
Acids		
Lactic, 1.0 N	69	8.9
Lactic, 0.5 N	46	9.1
Acetic, 1.0 N	40	9.2
Malic, 0.5 N	25	9.3
Oxalic, 1.0 N	23	9.4
Sulfuric, 0.1 N	16	9.3
Tannic, 0.5 per cent	13	9.3
Benzoic, saturated	12	9.4
Glycolic, 0.5 N	4	9.4
Salicylic, saturated	1	9.4
Salts		
Acidic		
Sodium silicate, 10 per cent, and lactic acid, 1.0 N., pH 3		
Flash-heat treatment	61	9.1
Dipped at room temperature	48	9.0
Calcium lactate, 1.0 N., pH 4.0	41	9.3
Calcium lactate, 1.0 N., pH 6.0	40	9.4
Sodium lactate, 1.0 N., pH 4.0	39	9.1
Potassium aluminum sulfate, 2 M	3	9.4
Neutral		
Calcium chloride, 2 M	45	8.6
Basic		
Sodium silicate, 2 per cent	21	9.3
Sodium tetraborate, 2 per cent	11	9.4
Calcium hypochlorite, 2 per cent	8	9.4
Sodium hexo phosphate, 5 per cent	5	9.3
Sodium silicate, 10 per cent	4	9.4
Bases		
Sodium hydroxide, 0.1 N	21	9.4
Calcium hydroxide, saturated	—1	9.5
Untreated eggs	0	9.4

[1] Eggs held for 10 days at 37.5°C.(99.5°F.) and 60 per cent relative humidity. [2] These data show representative results obtained from treatment with 125 different agents. [3] The pH of the albumen in fresh eggs was about 7.7. [4] The viscosity was measured at 28°C. by means of a Stormer viscosimeter.

to water vapor. Immersing eggs in calcium chloride solution decreased the loss of water during subsequent storage but presented the objectionable quality of keeping the shell surface continuously moist.

Sealing Effect of Mineral Oil With Stearic and Lactic Acids: The two grades of paraffin-base mineral oil tested showed a relative impermeability to moisture of 85 to 88 per cent in repeated experiments. A one-per cent addition of stearic acid to the oil increased the relative impermeability

of the oil coating to 95 per cent. From 0.5 to 2.0 per cent of lactic acid was added to the oil and stearic acid, and the mixture was stirred vigorously to produce an emulsion. This emulsion coating had the very high relative impermeability to water vapor of 99 per cent. Some eggs treated with this oil emulsion showed no loss in weight during a 20-day period under these drastic holding conditions. The pH of the albumen from oil emulsion-treated eggs stored for both 10- and 20-day periods was no different from that of albumen from fresh eggs.

When 0.5 per cent of acetic acid was added to mineral oil, favorable results were also obtained; however, because of its pungent odor, a mixture of acetic acid and mineral oil was found to be disagreeable to handle.

DISCUSSION

Although the lactic acid treatment is not as efficient in sealing the pores as the oiling method, the calcium lactate formed in and over the pores of the egg shell is quite soluble in hot water and only slightly soluble in cold water. When a lactic acid-treated egg is immersed in hot water, the calcium lactate dissolves and the expanded gases in the egg are allowed to escape. Oil-dipped eggs, on the other hand, are more likely to crack when they are boiled.

In all these tests the appearance of the albumen and yolk of dissected eggs was closely correlated with the decrease in weight of the egg and with the increase in pH of the albumen. Where no loss in weight occurred even during a 20-day storage period under incubating conditions, the appearance of the yolk and albumen was no different from that of a fresh egg. That very little change took place in the almost completely sealed eggs was substantiated by the fact that the high-frequency conductivity readings, Romanoff and Hall (1944), of the oil emulsion-treated eggs increased only three per cent during a 20-day incubating period, while the conductivity readings of untreated eggs held under the same conditions increased 53 per cent. From these experiments it is indicated that if the shells of fresh eggs are made completely impervious to the passage of carbon dioxide and water vapor, these eggs may not have to be stored at low temperature and high humidity in order to preserve their fresh quality.

SUMMARY

From a comparison of the effects of various sealing agents upon the loss in weight of eggs and upon the change in pH of albumen, it was determined that treatment of eggs with an emulsion of stearic and lactic acids in mineral oil decreased the permeability of the eggshell to water vapor to about one per cent of its original value. The sealing ability of mineral oil was improved by small additions of stearic, lactic, or acetic acids.

Dipping shell eggs in normal lactic acid was effective in reducing their loss of water and in slowing down the change in pH of the albumen.

Changes in the visible characteristics of the albumen and yolk of stored, dissected eggs appeared to be related closely to the increase in hydroxyl-ion concentration of the albumen and to the loss in weight of the entire egg.

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A GAUGE TESTER FOR HOME PRESSURE CANNERS[1]

C. I. GUNNESS[2] AND C. R. FELLERS[3]
Agricultural Engineering and Food Technology Departments,
University of Massachusetts, Amherst, Massachusetts

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Processes for home-canned, nonacid foods have been worked out for definite processing temperatures, usually 115.6 or 121.1°C. (240 or 250°F.), which correspond to approximately 10 and 15 pounds steam pressure per square inch, respectively, when all air has been exhausted from the cooker. Home pressure canners are, at present, equipped with pressure gauges but not with thermometers, therefore it is essential that the pressure gauges indicate the correct pressure within the canner.

There are several types of pressure gauges or regulators for home canners. The self-venting type of regulator automatically adjusts the pressure by means of a weight balanced against the internal pressure. These regulators theoretically do not get out of adjustment. The dial-type pressure gauges, however, do get out of adjustment, and if they read too high, underprocessing and spoilage may result; on the other hand, if they read too low, overprocessing will occur. Therefore, it is necessary to test and adjust these pressure gauges periodically.

It is very simple to calibrate pressure gauges in the laboratory where standard gauges and air pressure are available, but there is a need for a dependable portable tester for field work. Such a unit has been designed and is described below.

PRINCIPLE AND OPERATION

The gauge tester described below has been designed for testing gauges used on pressure cookers at five and 10 pounds pressure. The tester consists primarily of a cylinder and a movable weighted piston connected to the gauge being tested by means of a hollow tube. The gauge is tested by comparing its reading with a fixed known air pressure built up in the system by varying the total weight of the movable piston.

The total weight of the piston and fixed weight is one pound. The diameter of the piston is 0.5045 inch, giving an area of 0.20 square inch; therefore the piston builds up an air pressure of five pounds per square inch. An auxiliary weight of one pound is added so that the gauges can be tested at 10 pounds pressure per square inch also.

In operation the piston should be covered with a film of fairly heavy oil. To test a gauge, pull the piston to its full extent to admit air to the system and then press the opening of the gauge over the open end of the copper tube and firmly down onto the rubber stopper to prevent leakage

[1] Contribution No. 634, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

[2] Formerly head, Department of Agricultural Engineering (deceased).

[3] Head, Department of Food Technology.

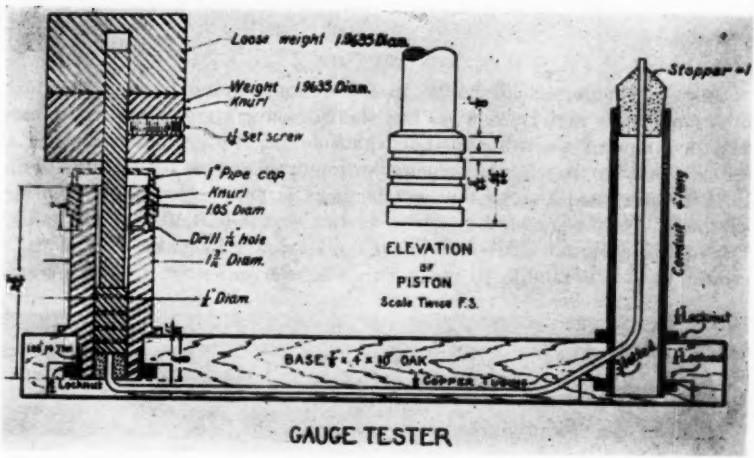

FIG. 1. Diagram of Gunness-Fellers gauge tester.

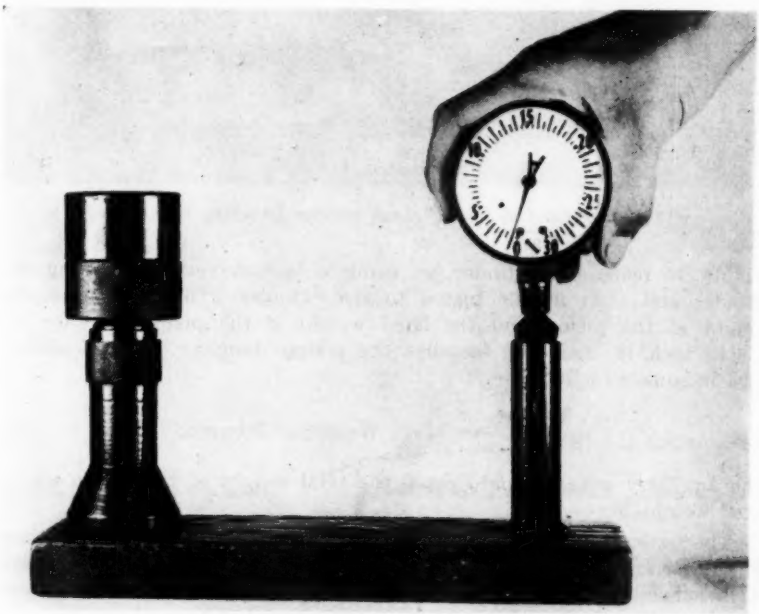

FIG. 2. Method of testing gauge (detached).

of air. Release the piston and spin it to prevent rapid leakage of air past the piston.

CONSTRUCTION

Construction details are given in the accompanying figure. The tester can be made in a variety of ways but the following suggestions are offered: It has been found desirable to drill the hole for the cylinder through the piece of stock so that the tester can be mounted on an arbor for turning and threading, and the bottom of the hole later can be plugged with a brass plug. It is suggested that the piston be made 0.5045 inch in diameter and the cylinder drilled, reamed, and lapped to make a good fit. If a reamer is not available to make this size conveniently, it will be satis-

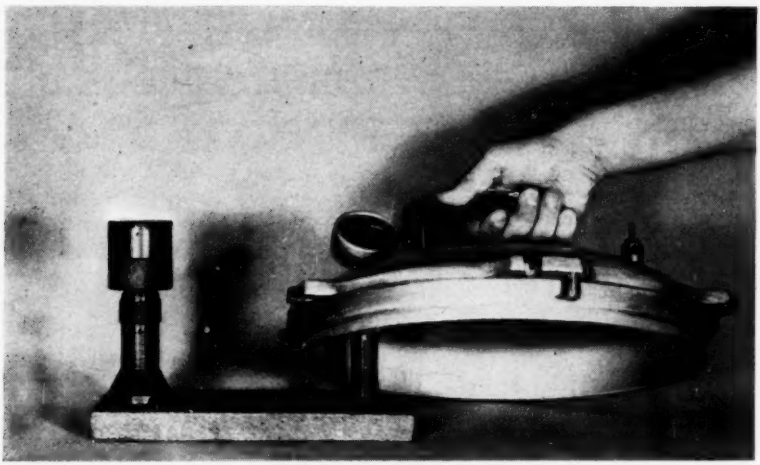

FIG. 3. Method of testing gauge without detaching from cover.

factory to make the cylinder by using a ½-inch reamer, lapping until smooth, and then fit the piston to the cylinder. To find the required weight of the piston and the fixed weight if the piston is other than 0.5045 inch in diameter, measure the piston diameter and calculate its area in square inches. Then:

$$\frac{\text{Weight}}{\text{Area}} = 5 \text{ lb.} \quad \text{Weight} = 5 \times \text{area.}$$

The auxiliary weight should equal the total weight of the piston and the fixed weight.

The tester is mounted on the wood base and held by locknuts as shown. The lower end of the cylinder is sealed with a brass plug through which a ⅛-inch hole has been drilled. A ⅛-inch copper tube is soldered into this hole and the tube laid in a groove cut in the wood base of the tester and brought up through a hole in the base as indicated. A four-inch piece of ½-inch conduit is threaded at one end and a slot ⅛ of an inch wide is cut on one side of the threaded portion of the conduit; this conduit is

slipped over the tubing and fastened to the base with another locknut at the bottom.

A No. 1 rubber stopper is drilled with a ⅛-inch drill and then tapered at the large end as indicated in the plate. The tubing is allowed to project up through the stopper for about ⅛ inch.

Credit is due W. C. Krueger of the New Jersey Agricultural Experiment Station who designed a compressed air-type tester with a long-contact tip. The long-contact tip is preferable because gauges can then be tested without removing them from the cooker cover. This avoids damage to the threads and leaky connections. We have found this device to be very useful.

The University of Massachusetts has constructed a number of these gauge testers for use by the County Extension officers. These are used to test gauges of home-type pressure canners and cookers locally and they eliminate the necessity of sending the equipment to the University. This saves much time and expense and also results in the calibration of more gauges each year than previously.

The instrument described above can be made in any machine shop at a cost ranging between $10 and $20.

BACTERIOLOGY OF SLIME IN A CITRUS-PROCESSING PLANT, WITH SPECIAL REFERENCE TO COLIFORMS

E. R. WOLFORD AND J. A. BERRY
Western Regional Research Laboratory,[1] Albany, California

[Received for publication, September 8, 1947]

Packers of orange juice to be marketed in frozen or chilled condition have come to attach importance to the numbers of bacteria in their product, and especially to its content of coliforms. Unless tests that differentiate between the sections of the coliform group are made, a juice may be considered unsatisfactory from the public-health viewpoint because of the presence of non-fecal coliforms that, under commercial conditions, are probably impossible to avoid. Wolford and Berry (1948) have shown that orange juice produced from good fruit even under laboratory conditions is not free from coliforms, while juice from "soft rot" oranges, in comparison with that reamed from sound fruit, yields markedly greater bacterial counts and coliform index. This paper deals with slime and débris on equipment in the juicing plant as a source of coliform and other bacteria that could appear in undue numbers in the finished juice.

Coliforms are ubiquitous, and their presence in slime is not peculiar to any type of plant. For example, Beckwith (1931) found *Aerobacter aerogenes* to predominate in slimes from paper pulp. He concluded that the source of the organisms was the water used. Martin and Dobson (1945) present similar evidence. Tonney and Noble (1932), in investigations of well waters, found coliforms growing on a wooden tank, a cheesecloth bag, a pump nozzle, fiber gaskets, graphited string packing, and leather washers. Spaulding (1931) and Caldwell and Parr (1933) report that some coliforms are able to grow on leather washers and leather packing. Spaulding, in addition, incriminated jute packing of water mains. Adams and Kingsbury (1937) and Calvert (1939) also found that jute could support growth of coliforms. According to Prescott, Winslow, and McCrady (1946), the organism usually responsible for such infections is *A. aerogenes*. *Escherichia freundii* occasionally occurs, but only on rare occasions is *Escherichia coli* involved.

Specific references to the bacteriology of citrus-processing equipment are few. Nolte and Von Loesecke (1940) found that water in washing tanks in two Florida citrus plants contained large numbers of *coli-aerogenes* organisms. The coliform content of raw juice from these plants ranged from 0 to 1,000 per milliliter. Teunisson and Hall (1947), in qualitative studies of the bacteriology of citrus canning, found *A. aerogenes* in cultures made from several places on the line, such as elevator rolls to scrubbers, knives of extractor, and the sizer belt.

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

EXPERIMENTAL PROCEDURE

Description of Line: In the plant selected for the studies the oranges were unloaded from semi-trailer trucks to an elevator, which took them to a receiving bin, whence they were conveyed by rubber belts to elevators leading to the storage bins. From the storage bins they were conveyed by means of belts and elevators to the sanders (machines which remove the flavedo by abrasion). From the sanders the oranges passed over a belt, an elevator, and another belt while being transported to the elevator leading to the sorting room. Conveyor belts took the oranges through the sorting room and the wash tanks. The washed fruit was lifted out of the water by bucket elevators to the scrubbers, where it was rinsed with clean water while passing over revolving brushes. After this treatment the oranges were conveyed over belts to juicing machines. The juice was flumed to defoamers and finishers and then piped through stainless-steel piping to the processing department.

Sampling: Samples of slime or other accumulation were taken from the points in the line indicated (Table 1). A sterile spatula was used to remove material from the line and transfer it to small sterile containers. One hundred-milliliter portions of the water in the wash tanks and of the juice were taken and placed in sterile jars.

Analysis of Samples: In the laboratory definite amounts of the samples were aseptically weighed into sterile containers, sterile water was added to give a dilution of 1:100, and the whole was well mixed. Further dilutions were planted on tryptone glucose extract agar, and on potato dextrose agar. The latter medium is considered favorable for detection of molds and yeasts. Counts were made after four days' incubation at 32°C.(89.6°F.). Formate ricinoleate broth was used for presumptive tests. Dilutions of wash-water and orange-juice samples were planted on the same media. Fermentation tubes showing gas in 48 hours or less were considered positive presumptive coliform tests, and eosin-methylene-blue plates were streaked from them. Colonies were fished from these plates to agar slants for later study. Data on total counts and presumptive tests for coliforms are given (Table 1).

Characteristics of Coliforms Isolated: Of 24 colonies fished from eosin-methylene-blue agar plates, 20 were gram-negative, nonsporing rods. One of the remaining organisms was a bacillus while the other three were yeasts. Special attention was given to the biochemical characteristics of the gram-negative bacteria. In addition to testing the organisms in 16 carbohydrate media, the following procedures were used: Barrit's (1936) alpha-naphthol method for determination of acetyl-methyl-carbinol (a variation of the Voges-Proskauer test); the standard methyl red test; Koser's test for utilization of citrate carbon; Kovak's indol test; Hajna and Perry's modification of the Eijkman test; and the boric-acid and high-temperature test of Vaughn and Levine (1935). In testing the ability of the bacteria to grow in orange juice, freshly reamed juice of Valencia oranges was sterilized by steaming for 20 minutes in small flasks and inoculated with a loop of broth culture. After incubation for five days at 30°C.(86°F.), smears were stained and examined under oil immer-

TABLE 1

Plate Counts and Presumptive Coliform Content of Samples of Slimes or Other Débris From Line in Citrus-Processing Plant

Sample No.	Point of sampling	Condition of line at sampling point	Total count in 1 gram (tryptone glucose extract agar)	Count in 1 gram (potato dextrose agar)	Smallest amount giving gas in fermentation tubes
					ml.
19	Elevator at unloading platform	Dry	378,000,000	278,000,000	1/2,000
20	Belt and elevators between receiving bins and storage bins	Dry	78,000,000	42,700,000	1/100
21	Scrapings from belt and elevator between storage bins and sanders	Moist	940,000,000	260,000,000	1/10,000
22	Scrapings from belt after sanders	Moist	157,000,000	135,000,000	1/10,000; small bubble in 1/20,000
23	Scrapings from elevator after sanders	Dry	540,000,000	224,000,000	1/100
24	Scrapings from elevator leading to sorting room	Dry, gummy	1,130,000,000	.....	1/2,000; Small bubble in 1/10,000
25	Slime from wash tank	Wet	3,240,000,000	850,000,000	1/200,000,000
26	Wash water	.....	29,000,000/ml.	5,000,000/ml.	1/10,000
27	Scrapings from belts leading to juicing machines	Moist	340,000,000	250,000,000	.....
28	Juice leaving machines	.....	160,000/ml.	50,000/ml.	.....
29	Finished juice	.....	570,000/ml.	480,000/ml.	1 of 2 tubes; 1/8,000

sion. On continued incubation, a ring of growth appeared in each positive flask at the surface of the juice. Data secured from all the tests are given (Table 2).

It is apparent that the organisms noted (Table 2) do not have the characteristics of *E. coli,* though two cultures, 29 and 30, fall in the intermediate class since they are methyl-red positive and do not produce acetylmethyl-carbinol but are able to utilize the citrate radical as the sole source of carbon. The balance of the organisms have IMVIC [2] reactions which place them in the *Aerobacter* section but often closer to *A. cloacae* than *A. aerogenes,* on the basis of ability to hydrolyze gelatin. Since Elrod (1942) and Stuart, Zimmerman, Baker, and Rustigian (1942) show the similarity between members of the genus *Erwinia,* pathogenic for plants, and the coliforms, inoculations of sound Valencia oranges were made with the organisms under study. Results were not conclusive. There was a tendency for the oranges to become moldy, and in the few cases where some softening was detected on mold-free fruit the changes were limited to the albedo peel and did not extend into the flesh of the orange.

DISCUSSION

When sanitation in a citrus-processing plant is imperfect, there may be considerable accumulation of slime on belts, elevators, and other equipment and in corners and crannies along the line. This slime harbors an extensive microbial flora and, especially when wet, undoubtedly contains many coliforms. In particular, the wash tank in the present investigation was in effect a culture medium for growth of microorganisms, and probably by itself made impossible the production of juice of low bacterial content. Operators have noticed that visible slime may accumulate in a wash tank a few hours after cleaning.

The bacterial numbers and incidence of coliforms found in the present investigation could no doubt have been greatly lessened by better sanitation, including perhaps chlorination of the water, which has been favorably reported by Hall and Blundell (1946) and Brownlee, Guse, and Murdoch (1947) in other types of food-processing plants. It is, however, to be noted that in this survey *E. coli* was not isolated either from the line or from the finished juice. The coliforms were predominantly *Aerobacter,* and while undesirable and to an undetermined extent avoidable, they have but limited significance from the public-health viewpoint. Hunter (1939) has pointed out the inapplicability of the coliform tests to such foods as milk and vegetable products because of the constant presence of coliforms. Moreover, the ease of confusion between coliforms and plant pathogens (*Erwinia*) serves further to detract from the usefulness of the routine analysis for detection of coliforms. It appears important, if coliform tests are used on raw-vegetable material at all, to determine whether the organisms isolated are IMVIC + + − −, or IMVIC − + − −, that is, whether they are *E. coli,* which is indicative of fecal contamination.

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[2] The term IMVIC was introduced by Parr (1939) and stands for the indol, methyl red, Voges-Proskauer, and citrate tests. The initial letter of the first three tests is used with Ci from citrate reversed for phonetic reasons.

TABLE 2

Biochemical Characteristics of Coliform Organisms Isolated From Points Along the Line in a Citrus-Processing Plant

Culture No.	Source[1]	Indole	Methyl red	Voges-Proskauer	Citrate	Uric acid	Eijkman, 46°C.	Boric acid, 44°C.	Gelatine liquefaction	Lactose	Sucrose	Maltose	Cellobiose	Arabinose
28	19	−[2]	−	+[2]	+	+	−	−	+	AG[3]	AG	AG	AG	AG
37	19	−	−	+	+	+	−	−	+	AG	AG	AG	AG	AG
29	20	+	+	−	+	+	−	−	+	AG	AG	AG	A	AG
30	20	+	+	−	+	+	−	−	+	AG	AG	AG	A	AG
31	21	−	−	+	+	+	−	−	+	AG	A	A	−	−
33	21	−	−	+	+	+	−	−	+	AG	AG	AG	AG	AG
34	22	−	−	+	+	+	−	−	+	AG	AG	AG	−	AG
44	24	−	−	+	+	+	−	−	−	AG	AG	AG	−	AG
35	25	−	−	+	+	+	−	−	+	AG	AG	AG	−	AG
36	25	−	−	+	+	+	−	−	+	AG	AG	AG	−	AG
38	25	−	−	+	+	+	−	−	+	AG	AG	AG	−	AG
39	25	−	−	+	+	+	−	−	−	AG	AG	AG	−	AG
43	25	−	−	+	+	+	−	−	−	AG	AG	AG	−	AG
45	25	−	−	+	+	+	−	−	−	AG	A	A	−	AG
46	25	−	−	+	+	+	−	−	−	AG	AG	A	AG	A
40	26	−	−	+	+	+	−	−	−	AG	AG	AG	AG	A
49	26	−	−	+	+	+	−	−	−	AG	AG	AG	AG	A
48	29	−	−	+	+	+	−	−	+	AG	AG	AG	AG	A
53	29	−	−	+	+	+	−	−	+	AG	AG	AG	AG	A
54	29	−	−	+	+	+	−	−	−	AG	AG	AG	AG	A

TABLE 2 *(Concluded)*

Culture No.	Source[1]	Rhamnose	Dextrose	Levulose	Galactose	Starch	Mannitol	Inulin	Dextrin	Glycerol	Inositol	Salicin	Growth in orange juice pH 3.84
28	19	AG	AG	AG	AG	—	AG	A[3]	—	—	—	AG	+
37	19	AG	AG	AG	AG	—	AG	AG	—	—	—	A	+
29	20	AG	AG	AG	AG	—	AG	AG	—	AG	—	—	—
30	20	AG	AG	AG	AG	A	AG	AG	—	AG	—	—	—
31	21	—	AG	A	AG	—	A	—	—	—	—	—	—
33	21	—	AG	AG	AG	A	AG	A	—	—	—	A	—
34	22	—	AG	AG	AG	—	AG	A	—	AG	A	AG	+
44	24	—	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	+
35	25	—	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	—
36	25	—	AG	AG	AG	—	AG	A	AG	AG	AG	AG	+
38	25	—	AG	AG	AG	—	AG	A	AG	AG	AG	AG	+
39	25	—	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	+
43	25	—	AG	AG	AG	—	AG	—	AG	AG	AG	AG	+
45	25	—	AG	AG	AG	—	A	AG	AG	—	—	A	+
46	25	—	AG	AG	AG	—	AG	AG	AG	AG	AG	AG	—
40	26	—	AG	AG	AG	AG	A	AG	AG	AG	AG	AG	+
49	26	—	AG	AG	AG	AG	AG	AG	AG	AG	—	AG	—
48	29	—	AG	AG	AG	AG	A	AG	A	—	—	A	—
53	29	—	AG	AG	AG	A	AG	—	A	—	—	—	—
54	29	—	AG	AG	AG	—	AG	—	A	A	—	—	—

[1] See Table 1. [2] —, no reaction; +, positive test. [3] AG, acid and gas in fermentation tubes; A, acid, no gas in fermentation tubes.

SUMMARY

Coliform organisms were abundant among the large numbers of bacteria present in slime and débris on belts, elevators, and wash tank in a citrus-processing plant. The number of organisms of all kinds tended to be greater where moisture was plentiful, reaching a maximum of over 3,000,000,000 per gram in slime from the wash tank.

Twenty coliforms isolated from six points on the line and from wash water and finished orange juice fell into two patterns: IMVIC − − + + (*Aerobacter*) and (in two cases only) IMVIC + + − + (intermediate). They therefore were not indicative of fecal contamination.

It would appear that routine tests for coliform organisms in orange juice are of limited significance. They should be supplemented by special procedures to detect members of the fecal (*Escherichia*) group to yield information of value from the public-health viewpoint.

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DETERMINATION OF PALATABILITY CHANGES OCCURRING IN FROZEN POULTRY [1]

GLADYS E. VAIL AND RALPH M. CONRAD [2]
Kansas Agricultural Experiment Station,
Manhattan, Kansas

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Within the past few years a whole new vocabulary has sprung into being with relation to foods. "Food acceptance" first became a widely used term during World War II when it began to be more fully recognized that no matter how adequate the diet provided, it was of value only when consumed and utilized. Other terms, more or less familiar prior to that time but more recently increasingly associated with foods, include "organoleptic tests," "palatability," "subjective testing," "taste panels," "the chemical senses," "olfaction," and "gustation." This emphasis on food acceptance may tend to confuse the picture, but in turn it has stimulated the workers to develop a system whereby foods may be more adequately evaluated.

At the present time the only really satisfactory method of determining the acceptance of a food is by the use of prospective consumers. The acceptability of a food to an individual is dependent upon many factors beyond the control of the investigator. Furthermore, the ability to recognize differences in foods is subject to individual peculiarities and is an exceedingly complicating factor in studying processing changes in any food. There is evidence, however, that persons with average ability to taste and smell foods may, with practice, learn to distinguish comparatively small differences.

"Taste and odor thresholds" as a measure of the ability of the individual to recognize taste and odor have been considered in many studies reported in the literature. King (1937) cited work in which a group of 64 individuals was tested. The threshold values for salt varied from 0.0008 to 0.2408 mole per liter; and for sugar, from 0.1004 to 0.1024 mole per liter. The ability of different individuals to recognize sour and bitter also varied widely. The statement is frequently made that there is no "taste blindness" comparable to "color blindness." However, Williams (1946) cited the incident of four persons tasting creatine. Three described it as "tasteless like chalk," while the fourth person who tasted it pronounced it bitter. Blakeslee (1935) reported the response of 6,377 people who tasted a solution of phenyl thiocarbamide. Of this number, 21.3 per cent recognized no taste and 65.4 per cent found it bitter. A few indicated that it was sour, others thought it was sweet, and still others said it was salty. Williams (1946) reported work with quinine, aspirin, saccharin, and various other substances which emphasized the individual differences

[1] Contribution No. 139, Department of Home Economics, No. 332, Department of Chemistry.

[2] Deceased.

in response to taste and tended to explain some of the inconsistencies obtained with subjective scoring of foods.

The sense of smell also varies greatly with individuals. This is probably, at least in part, an explanation of the selection of various cosmetics and toilet goods by different individuals. To what extent the ability to live in an atmosphere of stale and even unpleasant odor is due to odor fatigue and to what extent it is due to lack of olfactory acuteness is not known. It is a well-known fact, however, that certain otherwise normal people have practically no sense of smell, while others are able to recognize only certain odors with any degree of consistency. Blakeslee (1918) described his work with verbenas to show how individuals differ in their judgment of fragrance. Two verbenas were submitted to 40 persons. Ninety per cent noted fragrance in one or the other but not in both. Moncrieff (1946) explained this inconsistency as being due to partial anosmia (the condition of being unable to smell), and he believed it pointed to at least two kinds of smell receptors. Unlike taste, which according to various workers can be classified into four primary tastes—sweet, sour, bitter, and salt—for odors there is no simple classification acceptable to all workers.

Moncrieff (1946) listed 37 descriptions of smell and ended with "and so on." He also discussed the fact that few words are descriptive of smell as "red" is of color or "bitter" of taste. We have thousands of odors, hence the difficulty in accurately describing any one of them. While there are clearly differences between "bouquet" and "stench," the terms "ethereal" and "fragrant" may have no sharp-cut connotation for most people.

Moncrieff also stated that with smell as with taste we have little appreciation of intensity, and a 30-per cent change in intensity of stimulus is necessary to produce a perceptible difference in smell.

EXPERIMENTAL PROCEDURE

One of the problems long recognized by all persons concerned with the handling of foods is that of change during storage. Changes occurring in poultry in either cold or frozen storage have long been a problem, but early in the war the necessity of doing something to retard these changes became evident. Although chicken and turkey are usually high on the list of food likes of individuals and are not a regional food in any sense of the word, poultry served to the armed forces in the early forties often rated a low acceptance. It was noted that when subjected to identical storage conditions, some birds developed definite off-flavors while others remained of high quality. In an attempt to find reasons for this apparent discrepancy, work was begun some years ago in these laboratories. Organoleptic and chemical tests were run simultaneously on the same bird. This gave an excellent opportunity to study individual gustatory differences in the group serving as the palatability committee, and if possible to correlate organoleptic with chemical tests.

Dry-Heat Cookery: When the work was begun, adequate description for making organoleptic tests on poultry was not found in the literature, so preliminary exploratory work was done. Broilers had been selected for the long-time study to be started some months hence, so broiling seemed a

logical method to select for cooking inasmuch as no substance, such as water or seasoning, need be added for that cooking process.

The palatability committee was composed entirely of faculty members willing to give the necessary time to scoring the cooked birds. It was arranged that all members of the committee should be present by the time the first of a series of birds was cooked. The personnel of the committee included three from the Department of Poultry Husbandry, two from the Department of Chemistry, one from the Department of Dairy Husbandry, and one from the Department of Institutional Management. The composition of the committee remained constant for a period of two years; then changes began to occur gradually.

Some three months before the actual project was to begin, the training period for the committee was started. During this time various score cards were tried. After the birds were evaluated according to the score card being used, the card was discussed and criticized. The desirable parts of various cards were then combined to give what the committee considered an acceptable means of evaluation (Form I). The card included space for

FORM I

POULTRY COOKING RECORD

Grading Chart for Broilers

Use the accompanying key sheet and fill in each blank with a number which indicates the term that best describes the desirability or description of the factor being considered. Be sure each blank space is filled.

Sample No..... Date.....

Factor	Desirability	Description
Aroma		
Thigh, Color	xxxxxx	
Thigh, Flavor, lean		
Thigh, Flavor, skin		
Thigh, Tenderness		xxxxxx
Thigh, Juiciness		xxxxxx
Breast, Color	xxxxxx	
Breast, Flavor, lean		
Breast, Flavor, skin		
Breast, Tenderness		xxxxxx
Breast, Juiciness		xxxxxx

Signature.....

both desirability scores and descriptive terms. To help the members of the committee to recognize different forms of deterioration and also to develop an adequate descriptive vocabulary, chickens which had been in storage for various periods and under different conditions were cooked and evaluated. At first each person used the term which he considered to describe each factor best; these were then compiled and the results are shown (Form II).

Prior to cooking, frozen birds were defrosted in a refrigerator at 4.4°C. (40°F.) or less for approximately 40 hours. The broilers were cooked at a constant temperature of 148.9°C.(300°F.) until two thermometers, one in the breast and one in the thigh, had reached 87.8°C.(190°F.). When the second thermometer reached 190°F., the bird was removed from the

FORM II

KEY

For Use With Poultry Cooking Record

DESIRABILITY	DESCRIPTIVE TERMS
Aroma and Flavor	*Aroma and Flavor*
10. Extremely good	1. Flat
9. Very good	2. Faint
8. Good	3. Mild
7. Medium, plus	4. Mellowed
6. Medium	5. High, gamey
5. Medium, minus	6. Strong
4. Fair	7. Sharp
3. Poor	8. Rich
2. Very poor	9. Raw
1. Extremely poor	10. Old
	11. Oxidized
Tenderness	12. Bitter
10. Extremely tender	13. Foreign
9. Very tender	14. Fishy
8. Tender	15. Oily
7. Medium, plus	16. Acid or sour
6. Medium	17. Sweet
5. Medium, minus	18. Pronounced
4. Fair	19. Putrid
3. Tough	20. Normal
2. Very tough	21. Abnormal
1. Extremely tough	22. Rancid
Juiciness	*Color*
10. Extremely juicy	Thigh
9. Very juicy	1. Normal
8. Juicy	2. Abnormal
7. Medium, plus	
6. Medium	Breast
5. Medium, minus	1. Normal
4. Fair	2. Abnormal
3. Dry	
2. Very dry	
1. Extremely dry	

NOTE: If the color of either the thigh or breast is abnormal, describe with a note at bottom of score card.

oven and placed on a warm tray. The legs were disjointed and the main portion of the bird passed for each judge to evaluate the aroma, both as to desirability and description. In the meantime, the bones were removed and each thigh—muscles and skin—was cut crosswise into four pieces. Each judge received a corresponding sample from each bird tested. The breast muscles were then removed in two portions, and these in turn were cut into four samples each and given to the judges.

A plentiful supply of water and soda crackers was available between samples. An attempt was made to have the room light and pleasant, and talking was discouraged.

Approximately two years after the committee began to work together the evaluations obtained with a series of 14 types of frozen birds, tested at three-month intervals over a period of 18 months, were compiled and studied; the resulting scores are shown (Table 1).

TABLE 1

Average Scores for Broilers at Three-Month Intervals

Sample No.	Storage period (months)					
	3	6	9	12	15	18
1	51.6	55.3	55.4	61.7	58.9	53.8
2	54.7	56.3	50.1	49.3	56.5	56.0
3	50.2	59.7	57.4	57.3	55.0	49.6
4	56.2	50.1	40.4	56.9	51.8	53.0
5	53.3	59.8	56.7	60.7	53.8	56.8
6	59.3	52.0	55.1	65.0	61.5	53.0
7	56.8	59.0	60.3	46.9	53.8	58.4
8	49.3	64.1	61.6	56.3	65.5	57.2
9	60.1	58.9	56.7	57.0	61.3	51.8
10	55.3	58.7	57.1	58.9	55.7	58.6
11	54.6	62.3	58.4	59.9	64.5	57.2
12	57.3	59.3	54.1	53.9	59.8	59.4
13	68.1	55.0	62.0	54.7	56.0	59.8
14	59.6	61.1	55.4	52.1	57.0	59.0
Average	56.2	58.0	55.8	56.5	57.9	56.0

In numerical scoring the judges tended to follow individual patterns; that is, certain judges habitually gave high scores, whereas others tended to grade the birds down regardless of quality. Scores recorded were averages of the scores given by the individual members of the palatability committee. These scores (Table 1) do not indicate a consistent change in quality with increased storage. In fact, if one considers the average for the series of 14 birds at three-month intervals, it would seem that, as evaluated by the score card used, there was little change during the 15 months' period.

This may actually be the case; that is, for the conditions of this study the birds may have remained much the same for the entire period. On the other hand, it may be that individual judges found the birds to improve with storage while others scored them lower, with the resulting average score remaining about the same throughout the study.

An analysis of descriptive terms used shows that these were no more indicative of change than were the scores. The value of descriptive terms

is further questioned if one examines the number of times each term is applied to the birds at three-month intervals (Table 2). It is possible that the apparent decrease in oxidative flavor may be real, as it is said that the oxidative flavor of milk reaches a peak then tends to decrease with longer storage. This may be true of other foods also.

TABLE 2

Frequency With Which Each Descriptive Term Was Applied to Broilers at Three-Month Intervals

Term	Storage period (months)						
	0	3	6	9	12	15	18
Flat	25	27	39	33	30	34	36
Faint	15	21	18	21	36	26	10
Mild	47	86	56	72	59	78	44
Mellowed	23	56	37	53	38	17	20
High, gamey	6	15	0	6	3	3	3
Strong	12	29	13	20	17	20	15
Sharp	15	23	18	16	7	19	26
Rich	41	18	28	18	47	10	7
Raw	4	7	2	0	4	2	1
Old	16	53	56	78	63	66	44
Oxidized	34	74	59	73	41	42	55
Bitter	14	39	43	72	61	78	30
Foreign	14	18	41	34	51	36	19
Fishy	21	21	10	11	12	7	13
Oily	24	27	36	52	27	38	20
Acid	9	24	12	11	39	21	13
Sweet	3	5	8	11	13	2	0
Pronounced	2	0	5	2	0	0	0
Putrid	1	3	0	13	2	5	2
Normal	13	9	38	11	60	31	38
Abnormal	1	1	3	0	1	1	0

In an attempt to identify certain descriptive terms with certain persons, the frequency with which each term was used by different individuals was tabulated. No limit had been placed upon the number of terms to be used to describe any one characteristic. Some judges rather consistently used two or more terms to describe each characteristic while others found one term adequate, with few exceptions. This would be evident (Table 3) if one were to total the number of terms used by each judge.

Although "mild" was the term most often used, it was used consistently by all judges, not by any one person excessively. This is shown by the fact that it was among the six most-used terms of each of the judges. "Bitter," a term which has meaning for all, was frequently used by six judges; whereas four members of the palatability committee applied the term "oxidized" a great number of times. Two members used this term more frequently than any other, a third judge used it second in frequency, while a fourth judge used three other terms more. It was of interest that the four who used this term had either had previous experience in judging poultry or had worked extensively with oxidized foodstuffs and hence were familiar with the characteristics of oxidized fats. It would seem that the term oxidized has little meaning for the uninitiated. "Flat" was also

used frequently by four judges, and "old" and "oily" by three. Two members of the committee were largely responsible for the usage of the terms foreign, rich, normal, mellowed, sharp, and fishy, whereas only one used the terms sweet, strong, and acid a great number of times.

Considering that the judges scored portions of the same bird, it becomes quite apparent that a list of descriptive terms, even when worked out by the group itself, has little meaning in actual application. It is also quite evident that each member of the committee tended to use certain terms almost to the exclusion of others.

TABLE 3

Frequency With Which Each Descriptive Term Was Applied to Broilers by Individual Judges

Term	Judge A	Judge B	Judge C	Judge D	Judge E	Judge F	Judge G
Flat	41	37	11	19	53	54	5
Faint	10	12	2	4	7	60	48
Mild	45	58	42	76	32	68	150
Mellowed	11	6	72	72	28	17	44
High, gamey	2	7	6	1	1	21	1
Strong	18	11	16	15	17	8	40
Sharp	12	0	28	76	8	6	0
Rich	46	57	5	1	15	24	8
Raw	9	0	0	0	3	5	3
Old	40	41	29	17	58	30	161
Oxidized	19	107	1	1	64	130	53
Bitter	88	70	36	12	32	78	48
Foreign	83	1	2	121	10	4	1
Fishy	12	20	1	0	11	47	5
Oily	28	43	32	2	12	42	74
Acid	6	3	0	12	19	87	4
Sweet	9	0	25	1	2	5	0
Pronounced	1	1	5	0	2	1	0
Putrid	6	6	2	1	7	4	2
Normal	27	43	10	1	16	2	7
Abnormal	0	1	0	0	3	1	0

Examination of the use of terms by individual judges shows that eight terms are on both the most-used (six most frequently used terms by each judge) and the least-used (two or fewer times) lists, an added indication that the use of descriptive terms was not of particular significance in this study.

Moist-Heat Cookery: After 24 months the work was expanded to include hens. This necessitated the use of a different method of cooking. By this time the workers had begun to suspect that the high temperature reached on the surface when the birds were broiled might be responsible for failure to recognize any rancid fats present in the stored chickens, so it was considered advisable to try moist heat for cooking.

The hens were disjointed and cooked by simmering for three hours in tightly covered, heavy enamel kettles on surface burners in water equivalent to one-half the weight of the drawn bird. Salt equal to one per cent of the weight of the bird was used in the first cooking of the hens, then one-half of one per cent was tried; but in both instances certain members of

the palatability committee objected to the amount used, so it was omitted entirely for the remainder of the study.

The use of descriptive terms continued to be inconsistent. Oily was used more frequently, as might be expected, for many of the hens were exceedingly fat. However, the breast lean of one bird was described by the different judges as being mild, flat, rich, sweet, sharp, bitter, and acid. The terms applied to the aroma were somewhat more related, that of one bird being described as oily, foreign, oxidized, rancid, strong, old, and stale.

The numerical scores tended to be more consistent; that is, the hen which had the highest average score tended to be scored high by each judge, although there are many exceptions.

TABLE 4

Correlation Between Palatability Scores of Broiled Chickens and Results of Chemical Analyses for Peroxide and Aldehyde Values

Correlation between	Number of samples	Coefficient of correlation r
Odor score and logarithm of		
Skin aldehyde	66	—0.1232
Skin peroxide	66	0.0691
Internal aldehyde	66	0.0554
Total organoleptic score and logarithm of		
Skin aldehyde	66	—0.2401
Skin peroxide	66	—0.0363
Flavor and odor score and logarithm of		
Skin aldehyde	66	—0.2827[1]
Skin peroxide	66	0.1101
Internal aldehyde	66	0.0409

[1] Significant at five-per cent level.

Beginning in July, 1944, the young birds were also cooked in moist heat. The disjointed birds were placed in pottery casseroles just large enough to hold them; 240 c.c. of water was added and the casseroles covered. The birds were cooked in a 176.7°C.(350°F.) oven for one and one-half hours. Care was taken to have the pieces to be tasted, the thighs and breasts, in the center. Thus they were protected from direct contact with the hot casserole or the surface air, and any possible browning was avoided. No salt was added.

Although the results obtained by subjective testing were still far from satisfactory, statistical analyses were made of the data to determine any correlation which might exist between them and the chemical tests. Results with the broiled birds were disappointing. Even with the logarithmic values of the chemical data, which preliminary examination showed should be used since there was a curvilinear relationship between the organoleptic scores and the results of the chemical analysis, significant correlations were found in only one instance when the data for the first series of broiled chickens were analyzed (Table 4). Although the correlations are not significant, with the one exception, it is evident that the skin aldehyde is the chemical factor most closely related to the organoleptic scores.

In later work, when moist heat was used for cooking the chickens and a selected group of judges was used to evaluate them, much better correla-

tions were obtained (Table 5). As is shown by this table, there was a considerably higher correlation between scores for odor and chemical values than there was between scores for odor plus flavor and chemical values in the limited number of samples (20) for which both sets of data were available. The validity of odor alone as an organoleptic test of quality is indicated by the highly significant values obtained with a greater number of samples (Table 5).

TABLE 5

Correlation Between Palatability Scores of Chickens Cooked in Moist Heat and Results of Chemical Analyses for Peroxide and Aldehyde Values

Correlation between	Number of samples	Coefficient of correlation r
Odor and logarithm of skin peroxide	20	—.5297[1]
Flavor plus odor and logarithm of skin peroxide	20	—.3279
Odor and logarithm of skin aldehyde	20	—.5383[1]
Flavor plus odor and logarithm of skin aldehyde	20	—.3809
Odor and logarithm of skin peroxide	90	—.508[2]
Odor and logarithm of skin aldehyde	90	—.607[2]

[1] Significant. [2] Highly significant.

There are apparently several reasons why flavor plus odor scores do not show a higher correlation with chemical tests. Moncrieff (1946) believed that although odor has the greatest influence on flavor, such other factors as taste, roughness or smoothness, hotness or coldness, and pungency or blandness play a part. It is probable that in the case of the chicken, tenderness and juiciness also affected the judges unconsciously in the determination of the flavor score. Probably of even greater importance in determining the correlation of odor and flavor with chemical tests for aldehydes and peroxides is the effect of these substances on the chemical senses. Moncrieff reported that aldehydes are more reactive than alcohols, more volatile than acids, and have more interesting odors. It is only as the series of fatty aldehydes is ascended that the odor gradually changes from pungent, sharp, or disagreeable to pleasant. Many of the unsaturated aldehydes, such as acraldehyde or acrolein, have an intensely irritating odor. Aldehydes were no doubt responsible for odors frequently mentioned by one judge as being "ammonia-like." Peroxides are not listed by Moncrieff as being among the chemicals responsible for odors and tastes. He does state that some aldehydes are sweet but others are not; hence chemical changes responsible for odor changes might not be evident in taste or in flavor.

Since statistical analyses of the data indicated that the aroma was proving to be the best criteria for rancidity and that a highly significant correlation might be expected between the aroma score and the results of the chemical tests for aldehydes and peroxides, a new method of testing was adopted, based entirely upon aroma. This decision may be criticized on the basis that the method was arbitrarily selected to correlate with the results of the chemical analyses. On the other hand, the authors feel that it was well justified since (1) the purpose of the study was to follow the development of rancidity; (2) the total organoleptic score unquestionably includes many factors which are not related to rancidity; and (3) it was

almost universally true, in the birds studied, that aroma was the limiting factor in acceptability; that is, birds which would have been rejected because of poor aroma were frequently found to possess an acceptable flavor (aside from aroma) while birds with a good aroma were never found to have a seriously objectionable flavor.

After the test period in July, 1945, the aroma only was considered as far as the organoleptic tests were concerned. The individual birds to be tested were cooked in moist heat as described above. They were removed from the heat, and each judge smelled the bird, removing the cover only long enough to whiff the steam. About 15 minutes after removal from the oven, the birds were again tested for odor; then the covers were removed from the containers, the birds cooled for about five minutes, and once more evaluated for odor. The aroma, after the chicken was uncovered for a few minutes, was believed to correspond more nearly to that which would be present at the time of eating, and it largely determined the final aroma score.

The highly significant correlation between the chemical tests and the odor score would seem to justify the decision to use only odor as an organoleptic test for cooked poultry. When odor alone is evaluated, the variations encountered in sampling for tasting are not present, for all judges smell the same sample. In smelling, a few minutes between samples seems to be sufficient to overcome any fatigue experienced by the olfactory organs. The judging may be done quickly with a minimum of effort on the part of judges and research workers. It would also seem to be easier to learn to evaluate odor alone than it is to consider all factors which go into the determining of flavor. Furthermore, any change in the chemical nature of the fat may not be reflected in the tenderness and juiciness of the meat; hence odor would seem to be a valid instrument for measuring organoleptic changes caused by rancidity.

SUMMARY

A long-time study of storage changes occurring in frozen poultry gave opportunity for an extensive study of the characteristics of a palatability committee. It also provided data on chemical changes which might be correlated with palatability scores.

Methods for evaluating the palatability changes in poultry have been described.

Cooking in dry heat (broiling) with resulting browning and development of new odors and flavors was discarded in favor of moist-heat cookery, which was believed to retain original odors and flavors present in the uncooked bird.

Individual members of the palatability committee showed great variation in the scoring of frozen poultry.

Evaluation of flavor changes by the use of a rather complete score card gave results which showed little or no correlation with the chemical tests.

A combined score of odor and flavor did not show so high a correlation with chemical tests for aldehydes and peroxides in the fat as did odor alone.

It was concluded that the simplest and most reliable method for determining the organoleptic changes which take place in frozen poultry during storage is to cook the bird in moist heat in a tightly covered container, and then evaluate the odor after the bird has cooled slightly.

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CHANGES IN SUGAR CONTENT OF RAW GREEN BEANS DURING STORAGE[1]

VICTOR WILLIAMS, LAURA M. FLYNN, AND A. G. HOGAN
Department of Agricultural Chemistry, College of Agriculture, University of Missouri, Columbia, Missouri

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During an earlier investigation on methods of storing green beans it was noted that when they were kept packed in cracked ice for three or four days they became sweeter in taste. This observation seemed sufficiently important to warrant an attempt to determine the nature of the chemical reactions that were responsible for the change.

Kertesz (1933) reported that the total sugar in green peas stored for 20 hours at 25°C.(77°F.) dropped from an initial value of 15.8 mg. down to 7.0 mg. per pea, but that the total sugar content of peas stored at 3°C. (37.4°F.) was unchanged. Wright, Peacock, Whiteman, and Whitman (1936) showed that the sugar content of potatoes stored at 10 to 15.6°C. (50 to 60°F.) remained fairly constant, but that the percentage of sugar in potatoes stored at lower temperatures progressively increased.

Barmore (1936) advanced the theory that there are only two reactions which bring about changes in the sugar content of potatoes: (1) respiration, or the oxidation of sugar to carbon dioxide and water, and (2) the transformation of starch to sugar and vice versa. If the temperature of storage is reduced, respiration is retarded and the sugar concentration is increased. When the storage temperature is higher, the relative rates of the two reactions are reversed and there is an increase in starch.

Several methods for the determination of sugars have been adapted for analysis of plant extracts. Picknett (1940) preferred the Shaffer-Somogyi to the Quisumbing-Thomas method for determining sugars in plant materials. His preference was based on economy, speed, and applicability to materials with a low sugar content. He reported that green beans contained 2.44 per cent of reducing sugars and 0.29 per cent of sucrose. Heinze and Murneek (1940) studied five methods of determining sugar in plant extracts and concluded that Bertrand's was the most accurate. They also found that the Shaffer-Somogyi method was almost as accurate and was more rapid and convenient. It was decided, therefore, to use the Shaffer-Somogyi method in this investigation.

EXPERIMENTAL PROCEDURE

Sampling: Approximately nine kg. of green beans (Tender Green variety) were picked on August 29, 1946, and stored overnight at 4°C. (39.2°F.). The beans were well mixed the following morning and from them 16 500-gram samples were prepared. The first and last samples were combined and put immediately into a refrigerator at —40°C.(—40°F.) as

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a composite to be analyzed as a zero-time control sample. The other samples were wrapped separately in cheesecloth. Seven were placed in a one-half bushel basket and allowed to stand at room temperature. The other seven were packed in cracked ice and stored in a closed, insulated ice refrigerator. The cracked ice around the beans was replenished as often as necessary to keep them iced continuously.

At approximately 24-hour intervals individual samples were taken from the beans stored at room temperature and from those stored in the cracked ice. They were washed in tap water, excess moisture was removed with cotton towels, they were allowed to air-dry approximately five minutes, and then transferred to waxed cardboard containers and stored at —40°F.

Three days after the last samples were stored they were removed from the refrigerator one sample at a time, broken into sections one to one and one-half inches long and mixed thoroughly. A 100-gram sample was weighed out to an accuracy of 0.1 gram, placed in a graduated cylinder, and enough 95 per cent ethyl alcohol was added to make a total volume of 300 c.c. Approximately 200 c.c. of 95 per cent alcohol was required. Rapid work made it possible to keep the beans in a frozen state until the alcohol was added. The beans and alcohol were transferred to a Waring blendor and the graduate rinsed with two 50-c.c. portions of 95 per cent alcohol, making a total volume of 400 c.c. This mixture was blended for five minutes at full speed. At the end of this time the speed was cut down and a 100-c.c. aliquot was taken. An inverted 100-c.c. pipette was used to transfer the suspension from the blendor to a graduated cylinder.

Extraction: The 100-c.c. aliquot was filtered through an 80 x 30 mm. paper extraction cone and the filtrate caught in an extraction flask (standard taper, 250-c.c., pyrex). The graduate was rinsed with two 25-c.c. portions of 95 per cent alcohol and the washings poured over the residue in the cone and combined with the filtrate. Using this solution, approximately 80 per cent ethyl alcohol, as the extraction liquid the extraction and clarification of the extracts was carried out in the manner described by Heinze and Murneek (1940). The clarified extracts were filtered into a 100-c.c. volumetric flask, brought to volume with distilled water, and stored at 1°C.(33.8°F.) until analyzed for reducing and total sugars.

ANALYTICAL PROCEDURE

1. *Solutions:* a. Reagent 50 [Shaffer and Somogyi (1933)].

b. Potassium iodide-potassium oxalate [Heinze and Murneek (1940)].

c. 1 N H_2SO_4.

d. 0.1 N sodium thiosulfate [Shaffer and Somogyi (1933)].

e. 0.02 N sodium thiosulfate was prepared by diluting Solution d.

f. Potassium iodate [Shaffer and Somogyi (1933)].

g. Soluble starch [Heinze and Murneek (1940)].

h. Saturated lead acetate was prepared by dissolving approximately 400 grams of neutral lead acetate in one liter of water.

i. Standard glucose was prepared by dissolving one gram of reagent quality anhydrous dextrose (Merck and Company) in five liters of distilled water.

j. Sucrose solution was prepared by dissolving one gram of sucrose (Difco) in 10 liters of distilled water.

k. Sucrase (invertase).

A very crude sucrase preparation was made from Fleischmann's baker's yeast by the following procedure. Fifty 0.5-ounce cakes of the fresh yeast were dried in a vacuum oven, then extracted with approximately 300 c.c. of distilled water. The solids were separated by centrifuging and were resuspended in 100 c.c. of water. The solids were again separated by centrifuging and the two supernatant liquids were combined and poured into an equal volume of acetone. The mixture was stirred, allowed to stand a few minutes, filtered on a Büchner funnel, and the precipitate was air-dried. Approximately one-third of the precipitate was used in making up 200 c.c. of enzyme solution. Standardization with the pure sucrose solution showed that one c.c. of this enzyme preparation would completely hydrolyze one mg. of sucrose in 22 hours at 37°C.(98.6°F.).

2. *Methods:* For this investigation Reagent 50 was made up to contain five grams of potassium iodide per liter, since Shaffer and Somogyi had reported that in this concentration it is more stable and also less sensitive to nonsugar-reducing substances. With this reagent the 15-minute heating period used was optimum only for glucose and fructose, therefore the terms "reducing sugars" and "total sugars" as used in this paper are not absolutely accurate. However, the hydrolyzed sucrose could be determined with this reagent and, since it seemed probable that a large proportion of all the reducing sugars present would react within the 15-minute heating period, the reagent was standardized against glucose and all calculations were made in terms of glucose.

The procedure for the determination of reducing sugars was a slight modification of that given by Heinze and Murneek (1940), and it is outlined briefly in the following paragraphs.

The bean extracts were diluted 1 to 5 so that each cubic centimeter was estimated to contain from 0.1 to 0.25 mg. of reducing sugars. Duplicate determinations were run on each extract.

For each test 10 c.c. of extract was pipetted into a 25 x 200 mm. pyrex test tube, 10 c.c. of Reagent 50 was added, and the contents mixed thoroughly. With every set of four unknowns two blanks and two standards were included. Blanks were prepared by using 10 c.c. of distilled water and standards were prepared by using 10 c.c. of glucose solution (0.2 mg. per c.c.) instead of the unknown extracts. Each tube was covered with a small porcelain crucible and placed in a vigorously boiling water bath for exactly 15 minutes. The tubes were then removed and cooled to 35°C. (95°F.).

When the tubes were cool, two c.c. of the potassium iodide-potassium oxalate solution and 10 c.c. of 1 N H_2SO_4 were added to each. The tubes were rotated to insure complete mixing of the contents and dissolution of the cuprous oxide, and were allowed to stand three to five minutes before they were titrated. In titrating, 0.02 N sodium thiosulfate was added until most of the reddish-brown color had disappeared. A few drops of the starch solution were then added and titration continued until the end point was reached as indicated by the disappearance of the starch-iodine color.

The procedure for the determination of total sugars (reducing sugars plus sucrose) is given in the following paragraphs.

Exactly five c.c. of an unknown extract and three c.c. of the sucrase preparation were pipetted into a sterile 25 x 200 mm. pyrex test tube containing one c.c. of toluene. The tubes were shaken to insure complete mixing, plugged with sterile cotton plugs, and incubated for 22 hours at 37°C. A blank on the enzyme was prepared by using three c.c. of the sucrase solution and five c.c. of distilled water. A sucrose blank was prepared by using five c.c. of the solution containing one mg. of sucrose per cubic centimeter and three c.c. of distilled water.

After incubation the contents of each tube were transferred to a 100-c.c. volumetric flask and diluted to the mark with distilled water. The total amount of reducing sugars present in 10-c.c. aliquots was determined by the procedure previously described.

Calculations: The difference between the titration values of the blanks and the titration values for the standards gave the volume of sodium thiosulfate equivalent to two mg. of glucose. From these values the glucose equivalent of the sodium thiosulfate was obtained, and in subsequent unknown samples the reducing sugars present were calculated in terms of glucose.

The amount of sucrose was calculated as the difference between the reducing sugars present before and after hydrolysis with sucrase.

RESULTS

The data (Table 1) show that after seven days' storage at room temperature the reducing sugar content of the beans had dropped from an original value of 1.92 down to 0.90 grams per 100 grams fresh weight. Under the same conditions the amount of sucrose was unchanged. When calculated on a fresh-weight basis, there was no change in the amount of reducing sugar in the beans that were stored for seven days in cracked ice. The sucrose content rose, however, from an original value of 0.40 up to 0.85 gram per 100 grams.

If these changes are followed on a dry-weight basis, they are more marked because the beans stored at room temperature lost moisture through respiration and evaporation while those stored in ice actually imbibed water. Calculated on a dry-weight basis, the reducing sugar content of the green beans stored for seven days at room temperature dropped from the original value of 16.52 to a final value of 6.50 grams per 100 grams. The sucrose content of the beans stored at room temperature also showed a slight downward trend. The amount of reducing sugars present in the iced beans remained constant during the seven days' storage while the sucrose content rose from the original value of 3.45 to a value of 8.17 grams per 100 grams of dry matter.

After seven days' storage at room temperature the total sugars (reducing sugars plus sucrose) had decreased from the original value of 2.32 down to a value of 1.22 grams per 100 grams of fresh beans. During the same period the total sugars in the iced beans rose from 2.32 to 2.65 grams per 100 grams. Calculated on a dry-weight basis, the total sugars in the beans stored for seven days at room temperature dropped

from an original value of 19.97 to a value of 8.81 per 100 grams, and the total sugars in the iced beans rose from 19.97 to 25.48 grams per 100 grams of dry matter.

TABLE 1

Sugar in Green Beans

Storage time	Fresh-weight basis					
	Total		Reducing		Sucrose	
	Room temp.	Iced	Room temp.	Iced	Room temp.	Iced
hr.	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*
0	2.32	2.32	1.92	1.92	.40	.40
21	2.80	2.30	2.18	1.88	.62	.42
49	1.92	2.48	1.46	1.97	.45	.51
74	2.33	2.50	1.71	1.95	.62	.55
98	1.94	2.47	1.52	1.76	.42	.71
122	1.85	2.43	1.44	1.76	.41	.67
146	1.58	2.75	1.09	1.99	.49	.76
168	1.22	2.65	0.90	1.80	.32	.85
Storage time	Dry-matter basis					
	Total		Reducing		Sucrose	
	Room temp.	Iced	Room temp.	Iced	Room temp.	Iced
hr.	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*	*gm./ 100 gm.*
0	19.97	19.97	16.52	16.52	3.45	3.45
21	22.97	19.52	17.85	15.96	4.12	3.56
49	16.12	22.22	12.32	17.65	3.80	4.57
74	17.00	21.32	12.47	16.70	4.53	4.62
98	14.61	22.74	11.44	16.20	3.17	6.54
122	14.24	23.43	11.09	17.14	3.15	6.29
146	13.61	27.17	9.39	19.66	4.22	7.51
168	8.81	25.48	6.50	17.31	2.31	8.17

SUMMARY

Green beans stored in cracked ice became sweeter after three or four days. Chemical tests showed that after seven days' storage the total sugar had risen from 2.32 to 2.65 grams per 100 grams of beans. Most of this increase was in the form of sucrose.

Sugar in beans stored at room temperature for the same period of time had dropped from 2.32 to 1.22 grams per 100 grams of beans. Most of the decrease was due to loss in reducing sugars, the sucrose content showing only a slight decline.

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MICROBIOLOGY OF CANNED CORN AND PEAS

CHARLES M. WILSON[1] AND FRED W. TANNER
Department of Bacteriology, University of Illinois, Urbana, Illinois

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Corn and peas are among the most popular vegetables preserved by canning. They are subject, however, to serious spoilage by thermophilic bacteria unless they are properly prepared, processed, and stored. As much information as possible is desirable on the origin of these troublesome bacteria, their control in the canning plant, their resistance to processing conditions, and behavior in processed cans in the warehouse. This investigation was carried out to throw light on these questions. Much work has been done on microbiology of spoiled foods but less on the normal sound products which appear on the market. Canned vegetables present an interesting problem since it is difficult to produce a surgically sterile can and have one which is merchantable. The time has come when it is desirable to know more about normal food products.

The fact that spoilage in canned vegetables is due to bacteria has been definitely known since Russell (1895) isolated the causative organisms responsible for an outbreak of spoilage in canned peas in Wisconsin. Much progress has been made in the meantime and many publications appear in the literature. Since they have been summarized by various authors, no attempt will be made to do so here. The fact that thermophilic bacteria were factors in spoilage of canned vegetables was not recognized until Barlow (1913a,b,c) investigated spoilage in corn in a canning plant at Gibson City, Illinois, and reported the presence of living thermophilic bacteria which developed only when the cans were improperly cooled and/or stored in hot warehouses. He described two types of spoilage due to thermophilic bacteria. One is known today as "flat sour," so designated because such spoilage is manifested by production of acid but no gas. The ends of the can, therefore, remain flat. The second is characterized not only by acid production but by large quantities of gas as well, which distend the cans so that they often burst. Barlow also encountered spoilage in which no viable organisms could be isolated. This condition was later termed "autosterilization" by Tanner, Echelberger, and Clark (1932). In this situation spoilage organisms, probably thermophilic bacteria, die out in presence of their own products of metabolism so that cultures made from contents of what appear to be spoiled cans remain sterile. The importance of Barlow's work seems to have been ignored until investigators in canning technology recognized the importance of thermophilic spoilage organisms in canned vegetables. Bigelow (1919, 1920a,b) warned canners of thermophilic spoilage, as did Bigelow and Esty (1920) and Esty and Williams (1920). A third type of spoilage caused by thermophilic bacteria not observed by Barlow was described later by Werkman and Weaver (1927).

[1]Formerly Capt. Q. M. C., U. S. Army Quartermaster Subsistence Research and Development Laboratory, Chicago, Ill.

It was characterized by production of much hydrogen sulfide. Owing to solubility of this gas, swelling of the container does not occur. The product, canned corn or peas, is darkened and the unpleasant odor of hydrogen sulfide is noticeable when the can is opened. The contents are often disintegrated. It is an insidious type of spoilage because it is not apparent until the can has been opened.

As soon as the importance of thermophilic bacteria in canned-food spoilage was firmly established, investigations were undertaken to determine their origin, what steps in the canning factory were important in their destruction, and what control in the can would be necessary in case they survived.

Cameron, Williams, and Thompson (1927, 1928) reported results of a series of bacteriological field studies carried out during the canning seasons of the two previous years. When spoilage of canned peas or corn resulted from "understerilization," the cause was found to be due to something apart from the condition of the raw product. It seemed probable that it resulted from accumulation of spores in the equipment of the canning factory; they found that sugar used in making brine could be an important source of thermophilic spoilage organisms. These field studies were the first of many which were to add much knowledge to the bacteriological aspects of canning. Cameron (1929) observed that during the canning season of 1928 spoilage hazards in canning plants were rare, but he found that blanching equipment in the canning of peas and the precooking apparatus in the canning of corn ofter permitted establishment of thermophilic contamination. Michael and Tanner (1936) reported the bacteriological data from the examination of 900 cans of cream-style corn; 24 per cent contained viable cells capable of growing at 55°C.(131°F.). Significance of plant machinery in the thermophile problem in canning plants has recently been reviewed by Lipske and Hubbard (1947). The observations reported above give solid foundation to efforts to improve plant sanitation.

The problem of thermophilic spoilage organisms in sugar has been discussed by numerous investigators. James (1928), Gordon (1929), Cameron (1930a), Cameron and Bigelow (1931), Cameron and Yesair (1931), Bigelow and Cameron (1932), and Hucker and Pederson (1942) have all confirmed the early fears with respect to sugar. Cameron and Bigelow (1931), of the National Canners' Association Research Laboratory, finally published bacteriological standards for sugar and laboratory methods for analyzing canning sugar for canned-food-spoilage bacteria. These standards were based on the experience and observations of that laboratory in studying the sugar problem. The use of sugar complying with the bacteriological standards just mentioned has aided, in a large measure, in reducing spoilage hazards in canned vegetables due to thermophilic organisms.

Other ingredients used in canning may also carry spores of thermophilic spoilage organisms, according to Clark and Tanner (1937), who investigated starch, commonly used as an ingredient in canning of cream-style corn. As in the case of sugar, they found that starch might contain many thermophilic bacteria which spoil canned foods. Yesair and Cameron (1942) reported that flours and cereals often added to certain canned foods

may harbor organisms of spoilage significance. Bacteriological standards and methods for examination of starch have been established by the National Canners' Association Research Laboratory for the guidance of food technologists.

EXPERIMENTAL PROCEDURE

Since relation of certain groups of bacteria to spoilage of canned foods has been proved, and since it is recognized today that they may not always be destroyed in the canning factory and may survive without harming the quality of the food, an attempt was made to determine the incidence of thermophilic spoilage organisms in the various pieces of equipment in the plant and especially in the processed cans. Samples were obtained in 72 corn-canning plants located in Illinois and Wisconsin during the canning season of 1944. During the 1945 canning season samples were obtained from approximately 200 corn canneries located in Illinois, Wisconsin, Minnesota, Maryland, Delaware, and Pennsylvania. These included samples of cream-style, whole-kernel, and vacuum-pack corn.

With the same purpose in mind, samples were taken from 60 pea-canning plants in Illinois and Wisconsin visited during the 1944 season. The next year samples were obtained from approximately 250 pea canneries located in the same states as mentioned above in connection with the corn canning.

Because thermophilic organisms thrive at temperatures in excess of 46°C.(115°F.) and their spores will resist 100°C.(212°F.) for many hours, samples were taken wherever possible from any and all canning equipment which was subjected to heat. This applied to the canning of both corn and peas.

Samples From Corn-Canning Plants: For sampling cream-style corn a clean, specially constructed, metal dipper to hold approximately 100 grams was used. The sample was placed in a clean No. 2 tin can to which 150 ml. of water was added and thoroughly mixed. Approximately 50 ml. of liquid was strained through a sterile cheesecloth under aseptic conditions into a large, sterile culture tube. The tubes were then placed in ice water and kept chilled until cultured. No effort was made to sterilize the sampling dipper or the can in which the corn and water were mixed, as experience had shown that thorough washing and rinsing in potable water would suffice to remove danger of thermophilic contamination. Before culturing, all samples were subjected to a temperature of 108°C.(227°F.) (approximately five pounds steam pressure) for 10 minutes. This treatment was intended to destroy all non-heat-resistant organisms which would not be a factor in spoilage of the product and which are ordinarily killed in the processing.

Actual points of sampling were as follows: (1) silkers, which yielded raw corn just after being removed from the cob; (2) batch mixers after brine and perhaps starch had been added; (3) preheaters; (4) brine; and (5) the unprocessed line or the finished product in the can just before processing. At the same time the above samples were taken at least 12 processed cans were taken as samples for incubation at 55°C.(131°F.). An attempt was made to obtain samples truly representative of the plant at

the time and which would correspond to the samples taken throughout the canning procedure.

Since canning of whole-kernel corn is a simpler procedure and fewer pieces of heated equipment are used, a smaller number of samples had to be taken within the plant. Samples were taken in a thoroughly washed No. 2 tin can. The can was half-filled with corn, covered with potable water, and mixed vigorously. Approximately 50 ml. of liquid was strained through sterile cheesecloth into a large, sterile culture tube. These tubes were then handled as just described until cultured. The samples were taken as follows: (1) from the filler before the corn entered the can; (2) brine from the reservoir attached to the filler; and (3) liquid removed from a can just prior to processing. Here again, at the time the above samples were taken, 12 processed cans were also obtained for incubation at 55°C.

The operation of canning vacuum-pack corn is very similar to that of whole-kernel corn. The main differences are the use of smaller cans, addition of a much smaller quantity of cold brine, and closing of cans under a rather high vacuum. The points of sampling and methods used were the same as for whole-kernel corn, as was the number of samples for incubation.

Samples From Pea-Canning Plants: Since the canning of peas requires different equipment and procedures, samples taken in connection with the pea-canning operation were from different places than in the corn-canning plants. Samples taken in the pea-canning plants were as follows: (1) water from the blanchers (overflow if any, otherwise a sample taken from inside the blancher); (2) a sample of washings from peas removed from the filler (these washings were obtained by half-filling a thoroughly washed No. 2 tin can with peas, covering them with potable water, and mixing vigorously; approximately 50 ml. of liquid were strained through sterile cheesecloth); (3) brine from the reservoir attached to the filler; and (4) approximately 50 ml. of liquor from a can just before processing. At the time the above samples were taken at least 12 processed cans were taken as samples for incubation, as was the case in corn-packing plants. All such samples were selected so as to be representative of the plant and pack.

Samples other than processed cans taken for incubation were placed in large, sterile culture tubes and kept chilled in ice until they were cultured. Before culturing they were first subjected to a temperature of 108°C. (approximately five pounds steam pressure) for 10 minutes in order to destroy any nonresistant organisms which might be present and which would ordinarily be destroyed in processing the product.

Examination of Samples: Bacteriological examination of the samples of peas and corn was carried out in accordance with the methods published by Cameron (1938) and reviewed by Tanner (1944).

All glassware was sterilized for at least one hour at 121°C.(250°F.). All media were sterilized at 121°C. for 30 minutes to one hour. As mentioned previously, all samples to be cultured were subjected to 108°C. (approximately five pounds steam pressure) for 10 minutes to destroy any nonresistant organisms which might have been present.

Media for detection of the three common types of thermophilic spoilage organisms were employed. For cultivation of the thermophilic

organisms producing acid and no gas, commonly called "flat sour" organisms, dextrose tryptone agar (containing brom cresol purple indicator) as prepared by Difco Laboratories was used. For the detection of thermophilic-anaerobic organisms not producing hydrogen sulfide, commonly called thermophilic anaerobes or "T.A.," a dextrose tryptone broth was used. Prior to use, unless it was freshly prepared, tubes of the medium were exhausted in free-flowing steam for about 20 minutes or by heating at 108°C. for 10 minutes. After inoculation the tubes were stratified with a sterile agar. For detection of thermophilic anaerobes producing hydrogen sulfide, commonly called sulfide organisms or "sulfur stinkers," a sulfite agar medium was employed.

The above media were all prepared in accordance with well-known formulae.

Culturing of Corn: For detection of flat sour-producing organisms in cream-style corn, duplicate plates of dextrose tryptone agar were inoculated with one-ml. quantities of sample. This small volume was necessary because the suspended material present tended to cloud the medium. For detection of thermophilic anaerobes not producing hydrogen sulfide two tubes of broth were used, one tube receiving one ml. of sample, the other tube, three ml. For detection of thermophilic anaerobes producing hydrogen sulfide two tubes of the sulfite agar were used; each tube was inoculated with three ml. of sample. In culturing samples of brine, three ml. of sample were inoculated into duplicate tubes or plates of the media. All cultures were incubated at 55°C. for 48 hours, except when the presence of large numbers of organisms made it necessary to examine the plates of dextrose tryptone agar in a shorter time.

Whole-kernel and vacuum-pack corn were cultured in exactly the same manner as the cream-style corn except that in the detection of flat-sour organisms the plates of dextrose tryptone agar were inoculated with one ml. and three ml. of samples. The time and temperature of incubation were the same.

Culturing of Peas: Samples taken from the pea-canning operation were handled and cultured similarly to the samples of whole-kernel and vacuum-pack corn; methods and quantities used were the same.

Incubation and Examination of Processed Samples: Examination of the incubated, processed cans of peas and corn was exactly the same. Usually six (but sometimes 12 to 24) processed cans of the same lot were placed in an incubator at 55°C. for four days. At the end of this time all cans were opened and examined for spoilage. (Occasionally a much-swollen can would be removed before this time.) For determination of flat-sour spoilage, a few drops of dilute brom cresol purple indicator were added to the contents of the can. The appearance of a purple color was considered to indicate that the contents were sound. Appearance of a yellow color with this indicator was considered as an indication of acid production and spoilage of the product by thermophilic bacteria.

For determination of spoilage caused by thermophilic anaerobes not producing hydrogen sulfide, swelling of the cans when incubated at 55°C., and the development of an acidity and perhaps a "cheesy" odor were taken as an indication of spoilage by these organisms.

Spoilage by thermophilic anaerobes producing hydrogen sulfide was indicated by production of hydrogen sulfide manifested by the characteristic odor and a discoloration or darkening of the product. No swelling of the can should be noted in this type of spoilage because of the solubility of the hydrogen sulfide in the liquid.

RESULTS OF CULTURES AND INCUBATIONS OF CANS

Cream-Style Corn: Results of the experimental work described above, with cream-style corn (Table 1), indicate that a small percentage of the samples of raw corn showed the presence of these organisms. Not quite nine per cent of the samples showed the presence of flat-sour organisms and slightly more than one per cent were found to contain spores of the thermophilic anaerobe not producing hydrogen sulfide. No evidence of thermophilic-sulfide spoilage bacteria was found at any of the sampling stations or in the canned corn.

A total of 358 samples of corn from the batch mixers was examined; of this number 54, or 15.08 per cent, were found to harbor spores of the flat-sour spoilage type. Because of its construction and use this equipment

TABLE 1

Incidence of Thermophilic Spoilage Bacteria in Cream-Style Corn at Various Stations in Canning Plants and in Processed Cans.

Types of samples and bacteria	Cream-style
Total number of *raw-corn* samples	158
Spores of flat-sour producers in	14 or 8.86%
Spores of anaerobes not producing H_2S in	2 or 1.26%
Spores of anaerobes producing H_2S in	0
Total number of *mixer* samples	358
Spores of flat-sour producers in	54 or 15.08%
Spores of anaerobes not producing H_2S in	8 or 2.23%
Spores of anaerobes producing H_2S in	0
Total number of *preheater* samples	463
Spores of flat-sour producers in	105 or 22.67%
Spores of anaerobes not producing H_2S in	14 or 3.02%
Spores of anaerobes producing H_2S in	0
Total number of *brine* samples	183
Spores of flat-sour producers in	31 or 16.93%
Spores of anaerobes not producing H_2S in	1 or 0.54%
Spores of anaerobes producing H_2S in	0
Total number of *unprocessed-line* samples	318
Spores of flat-sour producers in	78 or 24.52%
Spores of anaerobes not producing H_2S in	7 or 2.2%
Spores of anaerobes producing H_2S in	1 or 0.31%
Total number of *processed cans* incubated	3,080
Spoilage due to flat-sour producers in	263 or 8.53%
Spoilage due to anaerobes not producing H_2S in	13 or 0.42%
Spoilage due to anaerobes producing H_2S in	0

is always considered a possible source of contamination. Mixers are used to mix the corn, brine, and starch if it is added. They are usually operated at high temperatures, around 88°C.(190°F.), and enough material may be left in them after improper cleaning to support growth of spoilage organisms. The heat necessary for development of thermophilic organisms is usually supplied by an improperly closed steam valve. Such conditions were observed in our surveys. Evidence of thermophilic anaerobes was demonstrated in eight, or 2.23 per cent, of the samples while the sulfide-spoilage organisms were not isolated. Although it is not shown (Table 1), an interesting observation was made in connection with canneries in the eastern states. Addition of starch to the corn did not seem to be common practice and the presence of thermophiles in cream-style corn was exceedingly rare. Whether this was significant or just a coincidence was not determined.

From 463 samples taken from the preheaters, 22.67 per cent showed the presence of spores of flat sour-producing bacteria; only 14 samples, or 3.02 per cent, were found to contain thermophilic anaerobes; and again no sulfide-spoilage organisms were noted. The fairly high percentage of samples showing presence of thermophilic organisms is not unexpected in view of the construction and use of these units. Many preheaters were so constructed that it was impossible to clean them properly without dismantling, which is not practical during the rush of the canning season. Since they are also subjected to heat, they are always looked upon with suspicion as a possible source of thermophilic contamination.

Slightly less than 17 per cent of 183 samples of brine showed the presence of flat sour-producing bacteria. Only one sample was shown to contain thermophilic anaerobes and no sulfide spoilage-thermophilic bacteria were found. In several instances, not noted here, samples of sugar were found to contain large numbers of thermophilic spoilage organisms although it was often impossible to isolate the organisms after the brine was made. This suggests the presence of comparatively non-heat-resistant spores.

A total of 318 unprocessed-line samples were taken, of which 78, or 24.52 per cent, were found to harbor spores of flat sour-producing thermophiles. Only seven, or 2.2 per cent, contained thermophilic anaerobes; sulfide-spoilage organisms were demonstrated in one sample. That processing cannot be relied upon to remove all chance for spoilage due to thermophiles is shown by the data from the incubation of processed cans. Examination of over 3,000 processed cans yielded 8.53 per cent of flat-sour spoilage, less than one per cent (0.42) spoilage owing to thermophilic anaerobes, and none owing to sulfide-spoilage organisms.

The results secured with cream-style corn (Table 1) seem to indicate that raw, cut corn is not unduly seeded with thermophilic bacteria which cause troublesome spoilage, but that as the corn passes through the mixers and preheaters, these organisms may build up as they do in the brine. These results justify the continued effort by food technologists to arouse greater interest among canners in plant sanitation with greater attention to cleaning machines. Presence of viable organisms in about 10 per cent of processed cans indicates again the necessity for proper cooling and

storage of such cans. It has been recognized for a long time that such cans of food are satisfactory for human consumption and will remain sound under proper storage conditions.

Whole-Kernel Corn: A summary of results from the examination of whole-kernel corn canning (Table 2) demonstrates that spores of flat sour-producing bacteria were present in nearly 11 per cent of 354 samples of raw cut corn. Only 0.56 per cent showed the presence of thermophilic anaerobes and no sulfide spoilage-thermophilic bacteria were found.

TABLE 2

Incidence of Thermophilic Spoilage Bacteria in Whole-Kernel and Vacuum-Pack Corn at Various Stations in Canning Plants and in Processed Cans

Types of samples and bacteria	Whole-kernel	Vacuum-pack
Total number of *raw-corn* samples	354	124
Spores of flat-sour producers in	38 or 10.73%	2 or 1.61%
Spores of anaerobes not producing H_2S in	2 or 0.56%	1 or 0.80%
Spores of anaerobes producing H_2S in	0	0
Total number of *brine* samples	236	73
Spores of flat-sour producers in	19 or 8.05%	6 or 8.21%
Spores of anaerobes not producing H_2S in	0	0
Spores of anaerobes producing H_2S in	0	0
Total number of *unprocessed-line* samples	258	112
Spores of flat-sour producers in	81 or 31.39%	15 or 13.39%
Spores of anaerobes not producing H_2S in	4 or 1.55%	0
Spores of anaerobes producing H_2S in	0	0
Total number of *processed cans* incubated	3,124	1,073
Spoilage due to flat-sour producers in	259 or 8.29%	21 or 1.95%
Spoilage due to anaerobes not producing H_2S in	2 or 0.067%	3 or 0.27%
Spoilage due to anaerobes producing H_2S in	0	0

Examination of the brine to be used in the canning of whole-kernel corn revealed that 8.05 per cent of 236 samples contained spores of flat sour-producing thermophiles. Neither of the other two groups of thermophilic organisms could be cultured.

Unprocessed-line samples of peas revealed the presence of spores of flat sour-producing thermophiles in 31.39 per cent of 258 samples. This sharp increase in incidence of positive samples in raw corn and brine is difficult to explain. A build-up in the filler would seem to be indicated, but seldom does one find thermophilic incubation temperatures in the filler bowl in connection with whole-kernel corn. Only occasionally is heat present in the entire whole-kernel canning line other than in the operation using hot brine.

During both canning seasons 3,124 processed cans were incubated and examined. Of this number 259, or 8.29 per cent, showed flat-sour spoilage; only 0.067 per cent showed spoilage due to thermophilic anaerobes; and no sulfide spoilage occurred.

It is interesting to note that in approximately 3,000 incubated samples each of cream-style and whole-kernel corn incidence of flat-sour spoilage

was almost identical: 8.53 per cent in cream-style and 8.29 in whole-kernel corn. This might be unexpected since the procedures for canning cream-style corn and also the history of the product might suggest much more difficulty from thermophilic spoilage with it than with whole-kernel corn.

For the results secured with the vacuum-pack corn (Table 2) fewer samples were examined than was the case with the other types; the number of samples showing the presence of thermophilic spoilage organisms is also lower. From 124 samples of raw corn, spores of flat sour-producing thermophiles were demonstrated in two, or 1.61 per cent, with one sample showing the presence of thermophilic anaerobe spores. Sulfide-spoilage organisms were not demonstrated to be present.

Thermophilic bacteria producing flat-sour spoilage were cultured from 8.21 per cent of 73 samples of brine, and in 13.39 per cent of 112 unprocessed-line samples. Thermophilic anaerobes and sulfide spoilage-thermophilic bacteria were absent in both types of samples.

A comparatively small percentage of incubated, processed samples developed flat-sour spoilage, 1.95 per cent from 1,073 samples, while 0.27 per cent produced gas at the incubation temperature and were classified as being due to thermophilic anaerobes. No sulfide spoilage was seen.

Examination of Canned Peas: Results of investigation on occurrence of thermophilic spoilage organisms found in pea-canning operations are given (Table 3). Some of the data are quite interesting and would seem to indicate that something still remains to be done in control of these organisms. Bacteriological literature indicates that the blancher in the pea-canning operation is likely to be a source of contamination. In this investigation almost 89 per cent of 1,055 samples were shown to contain flat sour-producing organisms. Only 1.32 per cent harbored spores of thermophilic anaerobes and none yielded sulfide-spoilage organisms. Pea canners have been warned repeatedly of the hazards of contaminated blanchers and have been given simple remedial measures many times. Blanchers require constant and adequate attention lest they become a source of contamination for the entire pack. Our observations bear out these warnings and show again that this operation in the pea-canning plant must be looked upon as hazardous to the careless canner.

Contamination of peas with thermophilic spoilage organisms seems to occur on the outside of the peas. Adequate and thorough washing of peas as they come from the blancher will do much toward eliminating these organisms. The data presented tend to bear this out; flat sour-producing organisms were found in 33.52 per cent of 1,038 samples of washed peas. Thermophilic anaerobes were demonstrated in 0.19 per cent of the samples and no evidence of sulfide spoilage-thermophilic bacteria was seen.

Thermophilic contamination was found to be more prevalent in brine used in canning peas than in brine used in canning corn. Brine for peas harbored flat sour-producing organisms in 20.08 per cent of 473 samples, thermophilic anaerobes in 0.21 per cent, and sulfide spoilage-thermophilic bacteria were not demonstrated.

TABLE 3

Incidence of Thermophilic Spoilage Bacteria in Peas at Various Stations in Canning Plants and in Processed Cans

Types of samples and bacteria	Peas
Total number of *blancher-water* samples	1,055
Spores of flat-sour producers in	938 or 88.9%
Spores of anaerobes not producing H_2S in	14 or 1.32%
Spores of anaerobes producing H_2S in	0
Total number of *washed-pea* samples	1,038
Spores of flat-sour producers in	348 or 33.52%
Spores of anaerobes not producing H_2S in	2 or 0.19%
Spores of anaerobes producing H_2S in	0
Total number of *brine* samples	473
Spores of flat-sour producers in	95 or 20.08%
Spores of anaerobes not producing H_2S in	1 or 0.21%
Spores of anaerobes producing H_2S in	0
Total number of *unprocessed-line* samples	1,090
Spores of flat-sour producers in	463 or 42.47%
Spores of anaerobes not producing H_2S in	5 or 0.45%
Spores of anaerobes producing H_2S in	0
Total number of *processed cans* incubated	9,654
Spoilage due to flat-sour producers in	1,905 or 19.73%
Spoilage due to anaerobes not producing H_2S in	29 or 0.30%
Spoilage due to anaerobes producing H_2S in	1 or 0.01%

Unprocessed-line samples of peas showed a much higher per cent of flat-sour contamination than corresponding samples of corn. Flat-sour organisms could be recovered from 42.47 per cent of 1,090 samples. Less than 0.5 per sample of the samples contained spores of the thermophilic anaerobes and none contained sulfide-spoilage spores. It is apparent that the sterilization load on the pea canner is important because many thermophilic spoilage bacteria must be destroyed.

During both canning seasons a total of 9,654 processed cans of peas were incubated and of this number 1,905, or 19.73 per cent, became sour. In other words, practically one-fifth of the more than 9,000 cans contained viable spores of flat sour-producing bacteria. Thermophilic anaerobe spoilage developed in only 0.30 per cent and sulfide spoilage in 0.01 per cent.

Reports in the literature reveal that commercially canned foods are not necessarily sterile but may contain viable, dormant bacteria which will develop only when the products are stored under conditions favorable for them. The term "commercial sterility" has been used to describe canned foods of this type. Ball (1928) stated that the object in processing canned foods is to destroy the organism present which might cause spoilage. Although it is possible to completely destroy all organisms present in food, to do so would probably cause the food to be unsalable.

Bigelow and Cameron (1932) stated that thermophilic bacteria were economically the most important causes of spoilage resulting from understerilization. That is the main reason for the great interest of food technologists in them. Perhaps the importance of results in this report should be minimized because they represent conditions during a time when the man-power shortage seemed to be at its height and all manner of bad practices in operating procedures obtained in many areas. If, on the other hand, the results reported here represent unwillingness of the industry to accept advice and guidance on how to eliminate these spoilage hazards, which we doubt, then spoilage caused by thermophilic bacteria due to understerilization is still a real threat to the canner.

Since these spoilage organisms can be eliminated and reduced in numbers or controlled to a large extent by proper and judicious use of cold, clean water, proper and adequate cleaning of machinery, and careful processing, some may think that the absence or presence of these organisms should be taken as an indicator of good or bad housekeeping in the plants. Few investigations seem to have been carried out, results of which would answer this question.

Much progress has been made in eliminating and controlling thermophilic spoilage organisms by using ingredients, such as salt, sugar and starch, which have met the standards discussed above. These products are not sterile and the small numbers of allowable bacteria can mushroom into dangerous proportions if proper handling and treatment of equipment is not observed.

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SUMMARY AND CONCLUSIONS

In order to determine the origin and incidence of thermophilic, spore-forming bacteria which cause three serious types of spoilage in canned corn and peas, many samples were taken from canning plants in six states and subjected to bacteriological examination during the canning seasons of 1944 and 1945.

These investigations revealed that the thermophilic organisms, especially those of the flat sour-producing type, were present in a rather high percentage of samples of both peas and corn. Presence of thermophilic anaerobes not producing hydrogen sulfide and those producing hydrogen sulfide were rarely demonstrated in either product.

Raw corn to be used in cream-style canned corn contained spores of flat sour-producing thermophiles in less than nine per cent of samples. Incidence of these organisms in samples taken from various equipment and unprocessed cans ranged from 15 to 24.5 per cent. Incubation of processed cans at thermophilic temperatures allowed the development of flat-sour spoilage in 8.5 per cent.

Slightly less than 11 per cent of samples of raw corn to be canned as whole-kernel corn was found to harbor the flat-sour type of thermophile. These organisms were demonstrated in eight per cent of brine samples

and 31 per cent of unprocessed-line samples. Actual flat-sour spoilage was detected in slightly more than eight per cent of 3,124 incubated, processed cans.

Examination of vacuum-pack canning procedures revealed a comparatively low incidence of thermophilic contamination. Flat sour-producing organisms were found in 1.6 per cent of raw-corn samples, eight per cent of brine samples, and in 13 per cent of unprocessed-line samples. Incubation of 1,073 processed cans revealed flat-sour type of spoilage in 1.95 per cent.

Incidence of flat sour-type thermophiles in pea-canning operations was, in general, higher than that found in corn canning. These organisms were found in 88.9 per cent of more than 1,000 samples of water from blanching equipment, in 33.5 per cent of samples of washed peas (after blanching), in 20 per cent of brine samples, and in 42 per cent of unprocessed-line samples. Flat-sour type of spoilage developed in almost 20 per cent of 9,654 processed cans incubated at thermophilic temperatures for four days. As in corn canning, thermophilic anaerobes not producing hydrogen sulfide and those producing hydrogen sulfide were only rarely demonstrated.

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RUTIN IN GREEN ASPARAGUS

FLOYD DEEDS AND JAMES F. COUCH

Pharmacology Division and Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture

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Glass jars of home-canned asparagus have been observed to contain a yellow deposit. Campbell (1939) has reported some studies on the nature of this deposit. He found the material to consist of aggregates of fine, needle-like crystals which were soluble in hot water and alcohol but insoluble in ether and gave color reactions characteristic of flavone derivatives. Aqueous solution of the crystals was yellow and did not reduce Fehling's solution. After hydrolysis with dilute hydrochloric acid, a deep-yellow substance was precipitated. This product of hydrolysis was insoluble in hot water but was soluble in ether and cold alcohol. The filtrate, after removal of the water-insoluble precipitate, reduced Fehling's solution. These findings suggested that the substance was a flavone glucoside and substantiated the report of Nakaoki (1932) that a derivative of the flavone series is present in fresh asparagus.

Because of a current interest in rutin obtained from buckwheat [Couch, Krewson, Naghski, and Copley (1946)] and an investigation of its pharmacological properties, a few tests have been made to determine whether or not the pigment found in green asparagus by Campbell was rutin. A sample of the compound studied by Campbell was available for examination.

In a report on some pharmacological properties of rutin by Wilson, Mortarotti, and DeEds (1947) it has been shown that rutin, in a concentration which produces no effect *per se*, prolongs the relaxation of excised guinea-pig colon induced by epinephrine. These same authors have unreported data which show the ability of rutin to stimulate the excised frog heart. These two techniques have been used in a study of the compound obtained from asparagus. The ability of the asparagus product to prolong the action of epinephrine is clearly demonstrated (Fig. 1); the marked stimulation of the excised frog heart produced by the same flavone derivative is shown (Fig. 2).

Crystals of rutin obtained from buckwheat (Fig. 3) and crystals of the compound found in green asparagus (Fig. 4) have been examined under the polarizing microscope and found to have the same optical and crystallographic properties. The birefringence, sign of elongation, and color of the two crystalline products were the same.

A sample of the asparagus compound was examined spectrophotometrically with the following results:

Maxima near 3627Å and 2577Å; minima 2827Å and 2352Å;
$K_{362.5} = 31.9$, $K_{375.0} = 27.97$, ratio 0.877.

The corresponding figures for pure rutin are 3627Å and 2577Å; 2827Å and 2352Å; 32.55 and 28.46, ratio 0.875 [Porter, Brice, Copley, and Couch (1947)].

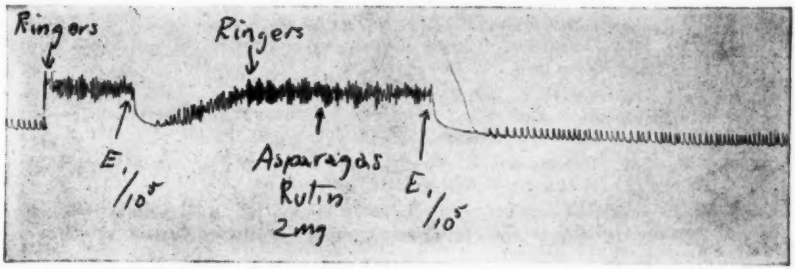

FIG. 1. Relaxation of excised guinea-pig colon by epinephrine, which is greatly prolonged in the presence of rutin obtained from asparagus.

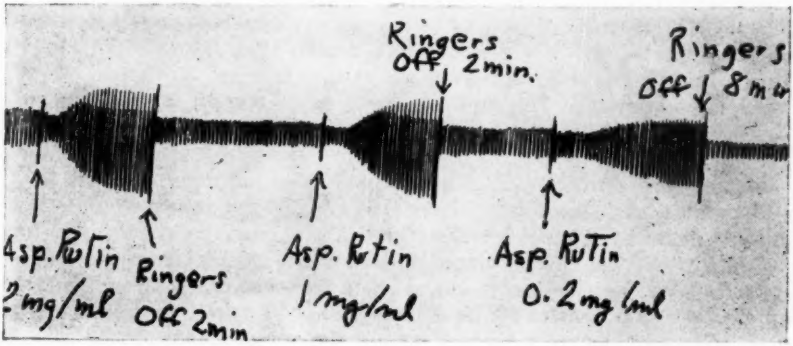

FIG. 2. Stimulation of excised frog heart by rutin obtained from asparagus.

FIG. 3. Crystals of rutin obtained from buckwheat.

FIG. 4. Crystals of rutin obtained from asparagus.

These data identify the yellow deposit as crude rutin. The optical measurements indicate a content of approximately 95 per cent rutin in the crude deposit.

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GAS FORMATION IN CONCENTRATED ORANGE JUICES AND ANALOGOUS SYNTHETIC MIXTURES [1]

A. LAURENCE CURL [2]
U. S. Citrus Products Station [3] Winter Haven, Florida

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Storage of pasteurized, concentrated orange juice at 26.7°C.(80°F.) and above, results in the formation of gas which has been shown to be essentially carbon dioxide. This gas does not result from fermentation. A number of possible sources of the carbon dioxide have been suggested, including the Maillard reaction between amino acids and sugars, decomposition or oxidation of ascorbic acid, and a chemical breakdown of sugars. A recent paper from this laboratory, Curl, Moore, Wiederhold, and Veldhuis (1946), included a literature review on this subject and also presented data showing that the formation of gas is accompanied by almost total losses of ascorbic acid, significant losses of total sugars, and considerable darkening.

In the work reported here, gas production was studied in concentrated orange juices containing added ascorbic acid, amino acids, or metallic salts and in synthetic mixtures containing various combinations of citric acid, potassium citrate, sugars, ascorbic acid, amino acids, etc., with the object of obtaining some information on the origin of the gas and the mechanism of gas formation. Some of the substances added to the orange juice were of such nature that they could not have any practical application in commercial practice. The samples were stored at 48.9°C.(120°F.) in order to accelerate the production of gas, earlier work by Curl (1947) having shown that the changes taking place in concentrated orange juice on storage at 80 and 120°F. are similar.

The samples were covered with mineral oil in test tubes provided with a device for collecting and measuring the volume of gas evolved. The possibility of gas formation by yeast or bacterial fermentation was minimized by the conditions of this investigation—a temperature of 120°F., a pH of about 3.7, and a sugar concentration of 50 per cent. There was no indication of any microbiological activity; duplicate and similar samples invariably yielded results in good agreement, and there were no erratic results.

EXPERIMENTAL PROCEDURE

Apparatus: The concentrated orange juices or synthetic mixtures in 25-gram portions were placed in test tubes of about 30-ml. capacity. Each test tube was fitted with a rubber stopper provided with an N-shaped piece of glass tubing of 5½ mm. inside diameter. The lower end of this tube was flush with the bottom of the stopper and was flared out to about

[1] Agricultural Chemical Research Division Contribution No. 214.

[2] Present address: Enzyme Research Laboratory, Western Regional Research Laboratory, Albany, California.

[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

10 mm. A corresponding part of the stopper was cut away, the purpose of this being to prevent the glass tube from being sealed off by the swelling of the rubber stopper in prolonged contact with mineral oil. The vertical arm of the tube extending through the stopper was about five cm. long, the other arms 10 to 12 cm. The test tube above the sample and all of the N-shaped glass tube excepting the upper portion of the outer vertical arm were filled with mineral oil so that no air was in contact with the sample. The upper bend of the N-shaped tube, in which the gas collected, had a capacity of about two ml., but was usually emptied when one-half to one ml. of gas had collected. The volume of gas was measured by noting the difference in the level of oil in the outer vertical arm before and after removing the gas by tilting the apparatus, and multiplying by the factor 0.025 to convert the millimeters to milliliters. The gas measurements, made at room temperature, were comparable and sufficiently accurate for the purpose of this investigation.

Materials: Most of the concentrated orange juices used were prepared in this laboratory (No. 1-6, Table 1); the others were of commercial origin. All of the concentrates had been prepared in connection with other investigations [Curl (1947a); Curl, Moore, Wiederhold, and Veldhuis (1946)] from pasteurized, single-strength juice and had been stored in cans at 4.4°C.(40°F.) until used. No. 1 to 6 contained 0.2 per cent of sodium benzoate.

In the synthetic mixtures 12.5 gm. (or 50 per cent) of sucrose, dextrose, levulose, invert sugar, or sorbitol was used. About half of the total sugars in orange juice is sucrose, and the remainder appears to approximate invert sugar in composition, Curl and Veldhuis (1947). The dextrose was anhydrous except in a few cases where an equivalent quantity of the hydrated dextrose was employed. The invert sugar consisted of equal parts of crystalline dextrose and levulose. Sorbitol was used in a number of samples since it is similar to the hexoses but contains no carbonyl group.

Five per cent of hydrated citric acid was used in all of the synthetic mixtures since it is the principal acid in citrus fruits. In the earlier work, where no buffer was used, the pH was about 1.8. In the later work 3.5 per cent of hydrated potassium citrate was added, bringing the pH to about 3.7, which is within the normal range of orange juice.

Nelson, Mottern, and Eddy (1933) found arginine, asparagine, and aspartic acid in orange juice. The first two were used in the present study, together with alanine (a typical monoamino-monobasic acid), cysteine, and cystine. The proteins—zein and edestin, the amides—acetamide and urea, the amines—benzidene and *m*-phenylenediamine, and ammonium sulfate were also used.

The substances—*l*-ascorbic acid, *l*-asparagine, *dl*-alanine, *d*-arginine hydrochloride, *l*-cystine, cysteine hydrochloride, and *m*-phenylenediamine hydrochloride—were actually used and are referred to hereafter in this paper as ascorbic acid, asparagine, alanine, arginine, cystine, cysteine, and *m*-phenylenediamine, respectively.

DISCUSSION

Concentrated Orange Juices: Results with concentrated orange juices with and without added ascorbic acid, amino acids, or metallic salts (Table

1) show that over a period of 60 days at 120°F. gas formation occurred in all concentrates having a soluble-solids content of 41 per cent and above (No. 3-9), while no gas formed in two samples of lower concentration (No. 1 and 2). In general, the amount of gas increased with increase in the soluble-solids content. In No. 9-11 copper sulfate, stannous chloride, and ferrous sulfate, respectively, were added to the concentrates. Addition of the first two salts resulted in a considerably increased gas production, but addition of ferrous sulfate caused no increase of gas. The addition of ascorbic acid increased the gas production markedly. In No. 13, where ascorbic acid was added to make its content five times the amount of ascorbic acid in the original sample, the volume of gas formed was about

TABLE 1

Gas Evolution From 25-Gram Samples of Concentrated Orange Juices on Storage in Glass at 48.9°C.(120°F.)

Sample No.	Soluble solids (by refractometer)	Ascorbic acid (original)	Volume of gas at room temperature over mineral oil	
			30 days	60 days
	pct.	*pct.*	*ml.*	*ml.*
1	20	.042	0.00	0.00
2	30	.067	0.00	0.00
3	41	.094	0.04	0.28
4	49	.111	0.15	0.63
5	60	.137	0.73	1.45
6	71	.161	3.08	9.25
7	65	.197	0.74	2.16
8	64	.206	0.99	2.91
9	No. 8 plus 0.4% $SnCl_2 \cdot 2H_2O$		1.62	4.57
10	No. 8 plus 0.4% $FeSO_4 \cdot 7H_2O$		0.97	2.17
11	No. 8 plus 0.4% $CuSO_4 \cdot 5H_2O$		1.67	.....
12	No. 5 plus 0.136% ascorbic acid		1.44	2.09
13	No. 5 plus 0.544% ascorbic acid		10.33	13.43
14	No. 5 plus 1.2% asparagine		0.86	3.14
15	No. 5 plus 1.2% arginine		0.65	1.64
16	No. 5 plus 1.2% alanine		0.61	1.47
17	No. 5 plus 4.0% alanine		2.03	9.25

10 times as great. The addition of 1.2 per cent of arginine or alanine (No. 15 and 16) had almost no effect, while the addition of the same amount of asparagine (No. 14) resulted in a slightly increased gas production in 30 days and about double that of the control sample in two months. Addition of four per cent of alanine (No. 17) increased the gas production to about three times that of the control sample in one month and six and one-half times in two months. Ascorbic acid was much more effective than amino acids in increasing gas production.

Synthetic Mixtures: Compositions of the synthetic mixtures used in these experiments and the amounts of gas formed (Table 2) indicate that no gas was formed in the absence of both ascorbic acid and nitrogen compounds (No. 18-21); added tin, ferrous, and copper salts were without effect (No. 22-24). Small amounts of gas were formed in mixtures containing sugars plus 0.2 per cent of ascorbic acid (No. 25-27), but no gas was evolved in the comparable mixture with sorbitol (No. 28). After a

TABLE 2

Gas Evolution From 25-Gram Samples of Synthetic Mixtures Analogous to Orange Juice on Storage in Glass at 48.9°C.(120°F.)[1]

Sample No.	Sugar	Other constituents	Volume of gas at room temperature over mineral oil	
			30 days	60 days
			ml.	*ml.*
18	Sucrose		0.00	0.00
19	Dextrose		0.00	0.00
20	Levulose		0.00	0.00
21	Sorbitol		0.00	0.00
22[2]	Dextrose	0.4% $SnCl_2 \cdot 2H_2O$	0.00	0.00
23[2]	Dextrose	0.4% $FeSO_4 \cdot 7H_2O$	0.00	0.00
24[2]	Dextrose	0.4% $CuSO_4 \cdot 5H_2O$	0.00	0.00
25	Sucrose	0.2% ascorbic acid	0.01	0.14
26	Dextrose	0.2% ascorbic acid	0.00	0.01
27	Levulose	0.2% ascorbic acid	trace	0.05
28	Sorbitol	0.2% ascorbic acid	0.00	0.00
29	Dextrose	2.0% ascorbic acid	6.34	20.94
30	Levulose	2.0% ascorbic acid	12.51	34.05
31[2]	Dextrose	0.2% ascorbic acid + 0.4% $SnCl_2 \cdot 2H_2O$	0.20	0.30
32[2]	Levulose	0.2% ascorbic acid + 0.4% $SnCl_2 \cdot 2H_2O$	0.20	0.78
33[2]	Dextrose	0.2% ascorbic acid + 0.4% $FeSO_4 \cdot 7H_2O$	0.10	0.15
34[2]	Levulose	0.2% ascorbic acid + 0.4% $FeSO_4 \cdot 7H_2O$	0.15	0.36
35[2]	Dextrose	0.2% ascorbic acid + 0.4% $CuSO_4 \cdot 5H_2O$	0.88	1.03
36[2]	Dextrose	0.2% ascorbic acid + 0.4% Cu_2O	2.52	2.87
37[2]	Dextrose	0.2% ascorbic acid + 0.4% CuO	0.73	0.94
38[2]	Dextrose	0.2% ascorbic acid + 0.4% $HgCl_2$	0.40	0.57
39	Dextrose	3% arginine	0.00	0.00
40	Dextrose	3% asparagine	0.02	2.62
41	Levulose	3% asparagine	0.00	0.18
42	Sorbitol	3% asparagine	0.00	0.00
43	Dextrose	3% alanine	0.00	0.28
44	Levulose	3% alanine	0.00	0.02
45[2]	Invert sugar	3% cysteine	0.03	1.52
46[2]	Invert sugar	3% cystine	0.00	0.00
47[2]	Invert sugar	3% zein	0.00	0.00
48[2]	Invert sugar	3% edestin	0.00	0.00
49	Dextrose	3% urea	0.09	0.14
50	Dextrose	3% *m*-phenylenediamine	0.00	0.00
51	Dextrose	0.2% ascorbic acid + 3% arginine	0.38	0.63
52	Dextrose	0.2% ascorbic acid + 3% asparagine	0.68	2.71
53	Dextrose	0.6% ascorbic acid + 3% asparagine	5.89	19.05
54	Dextrose	2.0% ascorbic acid + 3% asparagine	33.10+	46.98+
55	Levulose	0.2% ascorbic acid + 3% asparagine	0.28	0.71
56	Sorbitol	0.2% ascorbic acid + 3% asparagine	0.00	0.01
57	Dextrose	0.2% ascorbic acid + 3% alanine	0.50	1.13
58	Dextrose	0.6% ascorbic acid + 3% alanine	5.57	7.53
59	Dextrose	2.0% ascorbic acid + 3% alanine	34.32+	43.15+
60	Levulose	0.2% ascorbic acid + 3% alanine	0.24	0.58
61	Sorbitol	0.2% ascorbic acid + 3% alanine	0.00	0.01
62	Dextrose	0.2% ascorbic acid + 3% ammonium sulfate	0.33	0.46
63	Dextrose	0.2% ascorbic acid + 3% acetamide	0.08	0.28
64	Dextrose	0.2% ascorbic acid + 3% urea	0.38	0.79
65	Dextrose	0.2% ascorbic acid + 3% benzidene	0.17	0.55
66	Dextrose	0.2% ascorbic acid + 3% *m*-phenylenediamine	0.06	0.26

[1] Unless otherwise indicated all mixtures contained 50 per cent of a sugar, five per cent of citric acid (hydrate), and 3.5 per cent of potassium citrate (hydrate). [2] No potassium citrate added.

storage period of 60 days, 21 ml. of gas were evolved from the sample of dextrose plus two per cent ascorbic acid (No. 29), and 34 ml. were evolved from the sample of levulose containing the same amount of ascorbic acid (No. 30). When some compounds of tin, iron, copper, and mercury (No. 31-38) were added to the sugar and ascorbic acid mixtures, somewhat larger volumes of gas were evolved. A number of other metallic salts were tested, but, in general, only compounds of metals having more than one valence had much effect. The samples containing levulose (No. 32 and 34) evolved more gas than those containing dextrose (No. 31 and 33). This is in agreement with the results of storage studies of synthetic mixtures analogous to single-strength orange juice where ascorbic acid losses were larger with levulose than with the corresponding samples containing dextrose, Curl (1947b).

None of the mixtures containing both sugars and nitrogen compounds (No. 39-50) produced much gas in 30 days, but two mixtures (No. 40 and 45) yielded about two ml. in 60 days. With dextrose, asparagine (No. 40) gave much more gas than alanine (No. 43), while no gas was obtained with arginine (No. 39). With either asparagine or alanine, dextrose gave about 15 times as much gas as the corresponding samples containing levulose. This is the reverse of results with sugars and ascorbic acid. No gas was formed with invert sugar and cystine (No. 46), zein (No. 47), and edestin (No. 48). A small amount of gas was formed in the sample of dextrose plus three per cent of the amide, urea (No. 49), but no gas was evolved from the sample containing dextrose plus the same amount of *m*-phenylenediamine (No. 50).

Mixtures No. 51-66 contained sugars, ascorbic acid, and nitrogen compounds. These formed more gas than the corresponding mixtures containing no amino acids or no ascorbic acid. Results with the sample containing dextrose, 0.2 per cent ascorbic acid, and three per cent asparagine (No. 52) were similar to those with orange-concentrate samples (No. 7 and 8). The samples containing dextrose and asparagine yielded more gas than the corresponding samples containing alanine, which in turn yielded more than those containing arginine. Samples containing dextrose gave more gas than the corresponding samples containing levulose. Samples containing dextrose, two per cent ascorbic acid, and three per cent asparagine or alanine (No. 54 and 59) yielded considerably more gas than the sample containing no amino acid (No. 29). Gas production was so great in No. 54 and 59 that for several days some gas was apparently lost; the values given are probably low. In mixtures containing dextrose and ammonium sulfate (No. 62), dextrose and amides (No. 63 and 64), and dextrose and amines (No. 65 and 66) some gas was formed; this was considerably less than in the corresponding samples containing asparagine or alanine but significantly more than in the corresponding samples containing no nitrogen compounds.

Increase in color occurred in most of the samples during the two months' storage at 120°F. In no case did a very dark color form except in the presence of both a sugar and an amino acid or other nitrogen compound. The samples containing amines (No. 50, 65, and 66) developed very dark-red colors, the *m*-phenylenediamine forming a rubberlike gel.

Considerable darkening occurred with the amino acids, ammonium sulfate, and acetamide. Both of the samples containing urea were slightly colored. Samples containing ascorbic acid were generally darker than the corresponding ones without it, and samples containing asparagine were usually slightly darker than the corresponding ones containing alanine.

SUMMARY

A simple apparatus was devised for measuring the volume of gas evolved from concentrated orange juice or analogous synthetic mixtures in glass test tubes when stored at 120°F. The samples were kept under mineral oil, and the gas evolved was collected over the oil. A large number of concentrated orange juices and analogous synthetic mixtures were tested for gas production.

All samples of concentrated orange juice containing 41 per cent or more of soluble solids evolved gas on storage for 60 days at 120°F. Addition of ascorbic acid markedly increased gas production. Added amino acids had less effect, asparagine causing greater evolution of gas than alanine or arginine when present in equal amounts. Addition of stannous chloride or copper sulfate increased the production of gas.

The synthetic mixtures containing sugars with or without added stannous chloride, ferrous sulfate, or copper sulfate evolved no gas. Mixtures containing sugars plus 0.2 per cent ascorbic acid evolved only small amounts of gas, but when compounds of metals having more than one valence (Sn, Cu, Fe, Hg, etc.) were added to this mixture, gas production was considerably greater. Mixtures containing dextrose or levulose plus two per cent of ascorbic acid evolved considerable gas. Those containing sugars plus amino acids produced little gas in 30 days but considerably more in 60 days in some cases. Those containing asparagine produced more gas than the corresponding ones containing alanine, which in turn produced more than those containing arginine. Mixtures containing dextrose plus an amino acid yielded more gas than those containing levulose under the same conditions. Mixtures containing sugars plus ascorbic acid plus amino acids yielded gas in a quantity about the same as in concentrated orange juice. The most darkening was shown by mixtures containing both a sugar and amino acid or other nitrogen compound.

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COMPOSITION AND NUTRITIVE VALUE OF FRESH, COOKED, AND PROCESSED SWORDFISH [1]

ANTONIO LOPEZ-MATAS[2] AND CARL R. FELLERS
Food Technology Department, University of Massachusetts, Amherst, Massachusetts

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1. PROTEIN, FAT, MOISTURE, AND ASH CONTENT OF FRESH, FROZEN, DEHYDRATED, CANNED, AND BROILED SWORDFISH

The chemical methods used were those of the Association of Official Agricultural Chemists (1945).

All the samples employed were taken from the same specimen of fresh swordfish weighing about 300 pounds. This fish was bought in the wholesale market of Boston in July, 1946. The fish was iced but not frozen. Samples were taken for the determination of protein, fat, moisture, and ash in fresh swordfish. The rest was immediately placed in storage at 4°C. (39.2°F.) and within two days the preparation of the frozen, dehydrated, canned, and broiled swordfish samples was started.

The samples were prepared as follows:

(a) *Frozen Swordfish:* A piece of swordfish was boned, skinned, ground, and mixed in a Waring blendor. It was kept frozen in hermetically sealed glass containers at a temperature of —18°C.(—0.4°F.).

(b) *Dehydrated Swordfish:* When fish is commercially dehydrated it is usual to steam it prior to dehydration. This step accelerates the process by extracting water from the fish and by increasing the speed of dehydration; it was included in the preparation of this sample. In order to avoid mixing the water of the fish with condensed steam, the ground fish was placed in a 306 x 208 C enameled-type can and hermetically sealed. A thermometer was inserted in the can. After 90 minutes in a boiling-water bath the temperature at the center of the can was 94.5°C. (202°F.) The can was then water-cooled, and most of the free liquid was separated by pressing. The solids were placed in a drying cabinet, the fish temperature being maintained at 43 to 54°C.(109.4 to 129.2°F.) for the first five hours and raised to 66°C.(150.8°F.) for the last two hours. The liquid was concentrated to dryness on a water bath, which required 13 hours. The two dried fractions were finely ground and combined into a homogeneous mixture, which was stored in tightly closed glass containers at a temperature of —18°C.

(c) *Canned Swordfish:* Swordfish flesh was cut in small pieces, ground in a Waring blendor, and placed in 306 x 208 C enameled-type cans which were vacuum-sealed. The cans were processed in a retort for 70 minutes at 121°C.(249.8°F.) and water-cooled. Then they were opened and the contents mixed again in a Waring blendor. The homogeneous mixture was distributed in several small glass jars which were tightly closed and stored

[1] Contribution 630, Massachusetts Agricultural Experiment Station.

[2] Abstracted from a part of the Ph.D. thesis of A. Lopez-Matas, Fellow of the Corporaçion de Fomento of Chile.

at —18°C. Samples from a single jar were taken three times at most, in order to avoid differences owing to dehydration.

(d) *Broiled Swordfish:* Swordfish flesh was cut into steaks two inches thick. These were brushed with oil and placed on an oiled, shallow pan. They were cooked in a broiler on both sides for a total of 15 minutes, the temperature of the oven being 232°C.(449.6°F.). The fish was then cooled, ground, mixed, and placed in tightly closed glass jars. The storage temperature was —18°C. The protein, fat, moisture, and ash contents of the fresh, frozen, dehydrated, canned, and broiled samples of swordfish are given (Table 1).

TABLE 1

Protein, Fat, Moisture, and Ash Content of Fresh, Frozen, Dehydrated, Canned, and Broiled Swordfish [1]

Type of sample	Protein	Fat	Moisture	Ash
	pct.	*pct.*	*pct.*	*pct.*
Fresh	18.9	3.0	77.0	1.0
Frozen	18.7	3.1	76.6	1.0
Dehydrated	68.1	28.0	2.4	.....[2]
Canned	17.5	3.0	78.0	.....[2]
Broiled	28.7	5.1	64.5	.....[2]

[1] These determinations were made by the State Chemist's Laboratory, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts. [2] Not determined.

2. AMINO ACIDS IN FROZEN, DEHYDRATED, CANNED, AND BROILED SWORDFISH

EXPERIMENTAL PROCEDURE

In all cases microbiological methods were used, following the technique described by Kuiken, Norman, Lyman, Hale, and Blotter (1943). The bacteria cultures were obtained from the American Type Culture Collection, Georgetown University Medical School, Washington, D. C. Stab cultures were incubated at 37°C.(98.6°F.) for 48 hours and then kept in storage at 4°C. They were carried in duplicate, only one of the cultures being used for inoculations, the other being employed for the inoculation of new tubes every 10 days. Cultures for the assays were prepared by inoculating tubes of complete medium with the stab cultures, incubating them for 16 to 24 hours at 37°C., centrifuging, and suspending the cells in 10 c.c. of sterile saline solution. Each of the assay tubes was inoculated with one drop of this suspension. *Lactobacillus arabinosus* 17-5 was used in the determinations of isoleucine, valine, leucine, methionine, tryptophan, threonine, and phenylalanine; *Leuconostoc mesenteroides* P-60 for histidine and lysine; and *Streptococcus faecalis* for arginine.

RESULTS

Results of determinations in the four differently treated swordfish-flesh samples (Table 2) are expressed as the percentage content of each essential amino acid in the total protein of the sample. They are expressed in this way in order to be able to compare on the same basis the results obtained in the four different samples. The results expressed on a moisture-free basis would not be comparable because the broiled fish absorbed some oil during cooking, and this altered the relative content of the other

TABLE 2

Essential Amino Acid Content of Protein of Frozen, Dehydrated, Canned, and Broiled Swordfish Flesh

Amino acid	Frozen	Dehydrated	Canned	Broiled
	pct.	*pct.*	*pct.*	*pct.*
Arginine	9.1	7.6	9.6	8.8
Isoleucine	7.5	6.7	7.8	7.7
Histidine	3.3	2.8	2.9	2.9
Leucine	7.8	7.2	7.9	8.0
Lysine	7.2	6.8	7.0	7.1
Methionine	3.8	2.7	3.0	2.9
Phenylalanine	4.1	4.0	4.5	4.1
Threonine	2.6	2.2	2.1	2.6
Tryptophan	1.3	1.0	1.1	1.5
Valine	4.7	3.3	3.9	3.2

constituents. There were no gains or losses of protein in the four samples of cooked or processed swordfish. The results were calculated on basis of total weight (Table 3).

Comparing the essential amino acid content of the protein of swordfish flesh with that of beef, these amino acids account for 51.1 per cent of the total protein of swordfish and for 51.2 per cent of that of beef. The content of the individual amino acids is also very much the same. The main differences are observed in the threonine content, of which beef protein has 5.3 per cent and swordfish protein 2.6 per cent; in the histidine content, which is 2.1 per cent in beef protein and 3.3 per cent in swordfish protein; and in the arginine content, which is 7.2 per cent and 9.1 per cent, respectively.

TABLE 3

Essential Amino Acid Content of Frozen, Dehydrated, Canned, and Broiled Swordfish Flesh, on Basis of Total Composition

Amino acid	Frozen	Dehydrated	Canned	Broiled
	pct.	*pct.*	*pct.*	*pct.*
Arginine	1.70	5.20	1.70	1.50
Isoleucine	1.40	4.60	1.40	2.20
Histidine	0.62	1.90	0.50	0.84
Leucine	1.40	4.90	1.40	2.30
Lysine	1.30	4.60	1.20	2.00
Methionine	0.72	1.80	0.52	0.84
Phenylalanine	0.62	2.70	0.78	1.20
Threonine	0.49	1.50	0.36	0.75
Tryptophan	0.25	0.72	0.20	0.43
Valine	0.88	2.20	0.78	0.92

DISCUSSION

The results of these determinations show that canning and broiling of swordfish, compared with freezing, do not significantly affect the content of the several essential amino acids. The small differences found are attributed to human factors and to the laboratory methods used.

The amino acid content of the protein of dehydrated swordfish, for eight of the 10 essential amino acids, was significantly lower than that

for the frozen, canned, and broiled swordfish samples. For the other two amino acids it was the second lowest. As compared with the sample of frozen flesh, the average percentage retention of essential amino acids in the dehydrated swordfish sample is 85, while the same figure for both canned and broiled fish samples is 94. The interpretation given to these results is that dehydration caused small to moderate losses in all of the essential amino acids of swordfish flesh.

3. VITAMINS IN FROZEN, DEHYDRATED, CANNED, AND BROILED SWORDFISH

EXPERIMENTAL PROCEDURE

For vitamin A the method of Carr and Price (1926), as modified by Dann and Evelyn (1938), was employed. For thiamin, the thiochrome method was followed, as described by Hennessy (1942). Microbiological procedures were employed for riboflavin, Snell and Strong (1939); for nicotinic acid, Krehl, Strong, and Elvehjem (1943); for biotin, Shull, Hutchings, and Peterson (1942); for pantothenic acid, Strong, Feeney, and Earle (1941). For choline, the ammonium reineckate method of Engel (1942) was used.

RESULTS AND DISCUSSION OF VITAMIN DETERMINATIONS

Results are shown (Table 4) on basis of total weight, and (Table 5) on basis of amount of the particular sample equivalent to 100 grams of fresh swordfish. This latter way of expression was chosen in order to compare the effects of freezing, dehydration, canning, and broiling upon the vitamins investigated.

TABLE 4

Vitamin A, Thiamin, Riboflavin, Nicotinic Acid, and Biotin Content of Frozen, Dehydrated, Canned, and Broiled Swordfish Flesh [1]

Vitamin	Frozen	Dehydrated	Canned	Broiled
	mg.	*mg.*	*mg.*	*mg.*
Vitamin A	1,585(I.U.)	3,510(I.U.)	1,575(I.U.)	3,520(I.U.)
Thiamin	0.053	0.096	0.013	0.039
Riboflavin	0.049	0.334	0.048	0.052
Nicotinic acid	9.100	26.000	14.300	16.000
Biotin	0.0012	0.00036	0.00090	0.00075

[1] 100-gram portions.

TABLE 5

Comparative Effect of Freezing, Dehydration, Canning, and Broiling on Vitamin Content of Swordfish Muscle [1]

Vitamin	Frozen	Dehydrated	Canned	Broiled
	mg.	*mg.*	*mg.*	*mg.*
Vitamin A	1,585(I.U.)	965(I.U.)	1,680(I.U.)	2,290(I.U.)
Thiamin	0.053	0.026	0.013	0.025
Riboflavin	0.049	0.092	0.047	0.034
Nicotinic acid	9.100	7.100	15.300	10.000
Biotin	0.0012	0.0001	0.00095	0.00049
Pantothenic acid	0.050	.....[2]	.....[2]	.....[2]
Choline	250.000	.....[2]	.....[2]	.....[2]

[1] 100-gram portions; figures show the content of the amount of processed sample equivalent in weight to 100 grams of fresh swordfish muscle. [2] Not determined.

The low value for vitamin A found in the dehydrated swordfish sample was probably caused by oxidation of that vitamin during the process of dehydration and during storage. This reaction is accelerated with an increase in temperature, Rosenberg (1945). For the high values obtained in the broiled swordfish sample, no explanation can be offered at this time. The canned fish does not show any significant change in the content of this vitamin.

After the first determinations of thiamin, a very poor retention of this vitamin was observed. It was decided to study in more detail the effect upon this vitamin of different times and temperatures of processing. Results show that the destruction of thiamin is directly proportional to the length of processing (Table 6).

TABLE 6

Effect of Processing Time on Thiamin Content of Canned Swordfish at pH 6.2

Processing conditions	Thiamin	Retention
	μg./100 gm.	*pct.*
110 minutes at 121°C. (overprocessed)	0.0	0.0
70 minutes at 121°C.	9.5	18.0
90 minutes at 115°C.	13.0	24.5
40 minutes at 115°C. (underprocessed)	44.0	83.0
Control (frozen unprocessed swordfish)	53.0	100.00

The dehydrated and broiled fish samples show large losses of thiamin and the canned swordfish even larger, probably because of the instability of thiamin at high temperatures in a nearly neutral medium (pH 6.2). It is suggested that for better retention of thiamin the canned swordfish be acidified to pH 5.5, or as much as the flavor of the product permits.

In the canned sample no significant losses of riboflavin were detected. The broiled swordfish sample showed a serious loss, probably produced by the high temperature of broiling (232°C.). The dehydrated swordfish sample showed an apparent increase in the content of this vitamin. Several other workers have found apparent increases of riboflavin after the processing of different foods. Some suggest liberation of riboflavin during cooking from a complex that the microbiological assays do not include as riboflavin in the uncooked tissue, Hinman, *et al.* (1946). Others suggested microbial synthesis of riboflavin during storage of foods, Rice, Fried, and Hess (1946). In unpublished work the writers have found considerable increases in the bacterial counts of haddock during dehydration. There seems to be some possibility of the microbial synthesis of riboflavin during the dehydration process for fish.

The determination of nicotinic acid was first carried out using water extraction at pH 6.8 to 7. On canned swordfish this resulted in a retention of over 100 per cent. Then another determination was made in which the sample was extracted with normal hydrochloric acid solution and heating for 20 minutes at 121°C., according to the method of Krehl, *et al.* (1943). This method gave lower retentions (Table 7).

TABLE 7

Effect of Extraction Method on Nicotinic Acid Content of Swordfish Muscle

Method of extraction	Nicotinic acid content			
	Frozen	Dehydrated	Canned	Broiled
	mg.	*mg.*	*mg.*	*mg.*
Water, 30 minutes at 121°C.	6.5	5.2	12.8	9.6
Normal HCl solution, 20 minutes at 121°C.	9.1	7.1	15.3	10.6

The dehydrated and the broiled swordfish did not show significant changes in the nicotinic acid content as compared with the frozen swordfish sample. The canned swordfish showed a 168-per cent retention. The following explanation is offered. For the microbiological determinations of this vitamin the samples were extracted with water or diluted acid solutions at 121°C. The sample of canned swordfish was also processed in a tin can for 70 minutes at 121°C. The water naturally present in swordfish muscle probably acted as a supplemental extraction for this vitamin, for which reason a larger proportion of the nicotinic acid was made available for the bacteria used in the determinations.

Very low values were obtained for biotin when the sample was extracted with water during the autoclave treatment for 30 minutes at 121°C. The determination was repeated, modifying the extraction by following the method of Shull, *et al.* (1942), who used 3N sulfuric acid solution for one hour at 121°C. in an autoclave. The results (Table 8) show that the retention of biotin was high in the canned swordfish, small in the broiled swordfish sample, and almost nil in the dehydrated sample.

TABLE 8

Effect of Extraction Method on Biotin Content of Swordfish Muscle

Method of extraction	Biotin content			
	Frozen	Dehydrated	Canned	Broiled
	μg.	*μg.*	*μg.*	*μg.*
Water, 1 hour at 121°C.	45	22	43	13
3N sulfuric acid solution, 1 hour at 121°C.	1,200	100	950	490

4. GROWTH-PROMOTING VALUE OF SWORDFISH PROTEIN

This experiment was undertaken for the purpose of determining the adequacy for growth of white rats of swordfish protein, as compared with the proteins of beef and chicken.

EXPERIMENTAL PROCEDURE

The "paired" feeding technique was used, as described by Mitchell and Beadles (1930), modified in the composition of the diets as follows:

Mitchell and Beadles used the salt mixture of Osborne and Mendel (No. 1), butterfat, sucrose, and celluflour and starch as carbohydrate sources. In the experiments described herein the salt mixture No. 2 of the U.S.P. XII (1942) was used, which is a modification of the mixture

of Osborne and Mendel; corn oil was used as the source of fat; and starch was used as the source of carbohydrate. The above-mentioned authors used a protein level which ranged from 7.741 to 8.463 per cent, and a nitrogen level ranging from 1.239 to 1.354 per cent. In the experiments described herein the protein level of the diets was 8.00 per cent and the nitrogen level 1.28 per cent. Also, some synthetic vitamins were added which these authors did not include in their diets.

The animals used were albino rats, in groups of three. The rats in each group were litter mates, of the same sex, weighing approximately the same when the experiment was started. Each rat was kept in an individual cage with a raised-screen bottom, supplied with distilled water *ad libitum*, and with feed in a self-feeder. The rats were fed daily a definite weight of diet, with daily consumption of each rat kept at the level of the one eating the least. The total consumption of food of each group during the six or seven weeks of the experiment (some groups six, others seven) was approximately the same. A piece of paper was placed below the cages in order to be able to determine the amount of food they scattered; this amount was subtracted from the food supplied them in order to ascertain their daily intake. The composition of the diets is given (Table 9); the results are presented (Table 10 and Fig. 1).

TABLE 9

Composition of Swordfish, Beef, and Chicken Diets [1]

Component	Diets		
	Swordfish	Beef	Chicken
	pct.	*pct.*	*pct.*
Beef protein	.....	8.00	.....
Swordfish protein	8.00	.....	.....
Chicken protein	.....	.....	8.00
Beef fat	.....	0.74	.....
Swordfish fat	1.41	.....	.....
Chicken fat	.....	.....	2.16
Corn oil	9.30	8.90	9.84
Cod-liver oil (U.S.P.)	2.00	2.00	2.00
Starch	71.30	72.40	70.00
Salt mixture No. 2 (U.S.P. XII)	3.00	3.00	3.00
Agar	1.00	1.00	1.00
Yeast extract	2.00	2.00	2.00
Liver extract	2.00	2.00	2.00
Total	100.01	100.04	100.00

[1] In addition, the following synthetic vitamins were added per 10 grams of diet:

Thiamin	0.010 mg.
Riboflavin	0.020 mg.
Pyroxidine	0.030 mg.
Choline	2.000 mg.
Alpha-tocopherol	0.500 mg.
Calcium pantothenate	0.100 mg.

CONCLUSIONS

In these experiments the protein of swordfish and of chicken promoted about the same growth in rats, and both appear slightly superior to beef in this respect. The general conclusion can be drawn that the proteins

TABLE 10

Effect of Swordfish, Beef, and Chicken Protein in Food on Gain in Weight of Albino Rats

	Total diet intake	Total protein intake	Total gain in weight	Gain in weight per gram of protein intake
Group 1	*gm.*	*gm.*	*gm.*	*gm.*
Swordfish diet	442.6	35.4	118	3.34
Beef diet	442.5	35.4	104	2.94
Chicken diet	442.5	35.4	116	3.33
Group 2				
Swordfish diet	364.1	29.2	101	3.46
Beef diet	364.5	29.2	97	3.33
Chicken diet	365.0	29.2	107	3.66
Group 3				
Swordfish diet	380.9	30.5	104	3.42
Beef diet	382.5	30.5	91	2.98
Chicken diet	383.3	30.5	100	3.28
Group 4				
Swordfish diet	406.1	32.4	100	3.08
Beef diet	406.9	32.4	95	2.94
Chicken diet	406.7	32.4	104	3.22
Group 5				
Swordfish diet	438.0	35.0	112	3.21
Beef diet	438.1	35.0	111	3.17
Chicken diet	438.1	35.0	115	3.29
Group 6				
Swordfish diet	450.0	36.0	115	3.20
Beef diet	450.6	36.0	110	3.06
Chicken diet	450.5	36.0	120	3.34
Group 7				
Swordfish diet	442.6	35.3	99	2.81
Beef diet	442.6	35.3	93	2.64
Chicken diet	442.6	35.3	105	2.97
Average of all groups				
Swordfish diet	417.7	33.4	107.0	3.22
Beef diet	418.2	33.4	100.1	3.01
Chicken diet	418.4	33.4	109.5	3.30

of swordfish, beef, and chicken have about the same growth-promoting value for albino rats.

SUMMARY

The contents of arginine, isoleucine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine of the protein of frozen, dehydrated, canned, and broiled swordfish flesh were determined by microbiological methods. The ten essential amino acids were found present in swordfish in amounts very similar to those present in beef. Canning and broiling, as compared with freezing, did not significantly affect the essential amino-acid composition. The dehydrated sample showed losses which are considered significant.

The contents of vitamin A, thiamin, riboflavin, nicotinic acid, and biotin were determined in frozen, dehydrated, canned, and broiled swordfish. Pantothenic acid and choline were present in frozen swordfish in moderate to high amounts. Values of 1,585 I. U. of vitamin A, 0.053 mg.

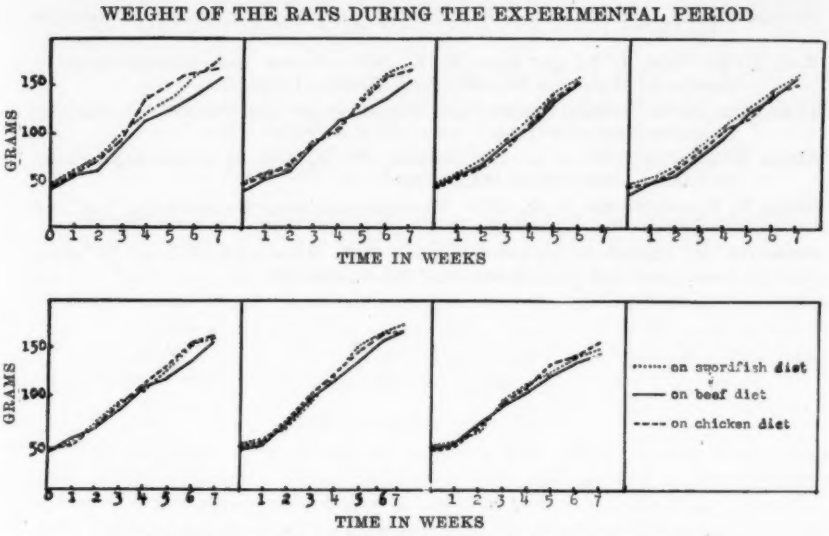

FIG. 1. Weight gains of individual rat trios for six to seven weeks.

of thiamin, and 9.1 mg. of nicotinic acid per 100 grams were obtained in the frozen swordfish. After the processes of dehydration, canning, and broiling, good retention was observed in general for vitamin A, riboflavin, and nicotinic acid and low retention for thiamin and biotin.

The growth-promoting values of the proteins of swordfish muscle, beef, and chicken were determined for albino rats, using the "paired" feeding technique. They were found to be approximately the same.

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VITAMIN CONTENT OF FIELD-PEA PRODUCTS: RETENTION OF THIAMIN AND RIBOFLAVIN ON COOKING[1]

HAZEL C. MURRAY

Department of Home Economics, Agricultural Experiment Station, State College of Washington, Pullman, Washington

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Outside of the rather complete work of Daniel and Norris (1945) on the vitamin content of the field pea *(Pisum sativum)*, few references are to be found in the literature on this subject. This is of considerable economic importance, since substantial amounts of field peas are being used in famine areas in Europe and Asia, as well as in low-cost dietaries in the United States. The cull pea also is proving of considerable value as a protein concentrate in livestock feeding.

EXPERIMENTAL PROCEDURE

Two varieties were examined—a green pea (Alaska) and the yellow pea (First and Best). The whole and split pea, as well as the fraction containing the germ, were assayed. (This fraction results from the splitting process; the product as obtained consists of about 40 per cent germ, the rest being made up of fragments of the cotyledon.) Samples were representative of two different crop years and were obtained from four processing plants.

The samples were ground and moisture determinations run. Thiamin was determined by the method of Conner and Straub (1941), modified to include extraction of the sample with iso-butyl alcohol, to remove non-thiochrome fluorescing material before conversion to thiochrome in lieu of adsorption, according to Harris and Wang (1941). The method of Peterson, Brady, and Shaw (1943) was used for riboflavin, modified by the use of tenth normal sulfuric acid for extraction. Autoclaving resulted in lower blanks, so it was used instead of boiling. Since a combination of papain and clarase for enzymatic digestion gave no higher results than clarase alone for both riboflavin and thiamin procedures, subsequent analyses were made with the latter only. The microbiological method of Snell and Wright (1941), using sodium hydroxide for extraction, as described by Teply, Strong, and Elvehjem (1942), was used for nicotinic acid. Carotene was determined by the method of Wall and Kelley (1943). Preliminary work showed that the carotene as well as the nicotinic acid content of the germ and split pea was much the same as that of the whole pea. These two determinations were, therefore, run only on the whole product.

Thiamin and riboflavin retention studies were made on boiled whole and split peas, also on baked and autoclaved whole peas.

RESULTS AND DISCUSSION

Values for the thiamin, riboflavin, nicotinic acid, and carotene assays are presented (Table 1). The thiamin and riboflavin figures for the split

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and whole pea are very similar. The germ proved somewhat richer in thiamin, and three to four times richer in riboflavin, than the endosperm, the assay for these two vitamins in the yellow germ being somewhat higher than that in the green. Peas possess only a tenth as much nicotinic acid as various meat products, such as skeletal muscle, heart, liver, and kidney, but are comparable to cereals and are a somewhat richer source of this material than fruits and other vegetables. The results for the

TABLE 1

Thiamin, Riboflavin, Nicotinic Acid, and Carotene Content of Pea Products

	Product	Number of samples [1]	Moisture	Range	Average
			pct.	*μg./gm.*	*μg./gm.*
Thiamin	Whole green peas	3	8.3	8.6-10.0	9.4
	Split green peas	1	7.2	9.8-10.0	9.9
	Green pea germ	3	7.4	11.1-12.0	11.5
	Whole yellow peas	3	8.5	10.7-12.0	11.4
	Split yellow peas	1	7.5	10.9-11.6	11.3
	Yellow pea germ	3	8.1	13.9-16.9	15.9
Riboflavin	Whole green peas	1	8.0	2.9- 2.9	2.9
	Split green peas	1	7.2	2.9- 3.0	3.0
	Green pea germ	1	7.0	8.0- 8.6	8.3
	Whole yellow peas	1	8.5	2.8- 3.1	2.9
	Split yellow peas	1	7.5	3.5- 3.5	3.5
	Yellow pea germ	1	8.8	9.0- 9.1	9.1
Nicotinic acid	Whole green peas	2	8.5	30.9-31.4	31.1
	Whole yellow peas	2	8.5	30.6-31.8	31.2
Carotene	Whole green peas	2	8.2	12.2-16.3	13.9
	Whole yellow peas	1	8.7	13.9-16.6	14.8

[1] Six duplicates were run on each sample.

carotene determinations indicate that the field pea is a poor source of this vitamin and that the yellow pea is not a better source than the green variety.

Values for thiamin and riboflavin from this study are somewhat higher than those of Daniel and Norris (1945), but the nicotinic acid content is quite comparable to that obtained by them.

Retention of thiamin and riboflavin on cooking by various methods is shown (Table 2). In all of these trials the peas were cooked sufficiently to give a desirable texture and flavor. Thiamin was somewhat lowered by cooking processes, the split peas in general suffering less loss owing to a shorter cooking period; baking and autoclaving were rather destructive. The riboflavin content of the pea showed a marked increase on cooking: the greatest increment resulted from longer cooking processes, such as boiling or baking for some time. Other workers have reported similar results. Hinman, Tucker, Jans, and Halliday (1946) and Richardson, Mayfield, and Hedrick (1945), working on beef, and McGregor (1944), working on pork and lamb, have shown that increases result on cooking. These findings indicate that the chemical method as employed on the

TABLE 2

Retention of Thiamin and Riboflavin in Peas Cooked by Various Methods

Product and Treatment	Moisture	Thiamin	Riboflavin	Retention	
				B_1	B_2
	pct.	*μg./gm.*	*μg./gm.*	*pct.*	*pct.*
Uncooked split pea	7.2	10.3	2.9	.....	.....
Split peas soaked 12 hr., 10 min. cooking	.....	10.2	3.3	99	114
Split peas unsoaked, 30 min. cooking	.....	9.4	3.9	91	134
Uncooked whole peas	7.3	9.0	3.5	93	.....
Whole peas soaked 12 hr.,30 min. cooking	.....	8.8	3.8	85	108
Whole peas baked, soaked 12 hr., boiled 15 min., baked 2 hr. at 190.6°C.(375°F.)	.....	7.5	4.5	73	129
Whole peas autoclaved, soaked 12 hr., autoclaved at 15 lb. pressure for 1 min	.....	7.0	.....	68	.....

raw product for riboflavin determinations does not give values representative of the total content.

SUMMARY

A study has been made of the thiamin, riboflavin, nicotinic acid, and carotene content of yellow and green field-pea products. The field pea is a very good source of thiamin and a fair source of riboflavin. The germ is somewhat richer in these vitamins than the endosperm. The nicotinic acid content is comparable to that of whole cereals.

The thiamin content is somewhat lowered by cooking processes; whereas, there is an apparent increase in riboflavin.

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EFFECT OF pH ON THERMAL PROCESS REQUIREMENTS OF CANNED FOODS

PETER SOGNEFEST, G. L. HAYS, EVAN WHEATON, AND H. A. BENJAMIN

Research Division, American Can Company, Maywood, Illinois

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To insure that canned food will remain wholesome, canning procedures incorporate the fundamental practices of hermetically sealing the container and sterilizing the product by heat. In determining the sterilizing process, it is necessary to know the heat resistance of bacterial spores which may cause spoilage and the rate at which heat penetrates the product during thermal processing. Methods developed by Bigelow, Bohart, Richardson, and Ball (1920); Ball (1923, 1928); Olson and Stevens (1939); and others are used in determining the process time and temperature.

Among the various factors affecting thermal resistance of bacterial spores or their ability to germinate, one of the most important, from the standpoint of canned food sterilization, is pH. This has been generally recognized for several decades, but there is little quantitative data to be found in the literature giving the specific effect of pH on commercial thermal death times in specific foods. Commercial thermal death times may be considered those times at which bacteria have lost the power to germinate at temperatures less than 37.8°C.(100°F.) in the food in which they were heated whether or not they have been killed (i.e., no spoilage occurs). The lack of these data has instigated the present work since there is apparently a real need for quantitative data of this sort.

Most of the thermal death time work in the past has been done in juices extracted from foods or in processed foods in glass thermal death time tubes. In these series of tests the suggestion by Townsend, Esty, and Baselt (1938) of employing raw foods rather than processed products has been followed, and in order to simulate commercial conditions as far as possible, small metal cans rather than glass thermal death time tubes have been used.

Spoilage organisms used in these studies were a putrefactive anaerobe isolated by the National Canners' Association Research Laboratories and known as N.C.A. 3679, and *Clostridium botulinum* strain 213, (Type B). Destruction of this latter organism is of particular concern to the canning industry because of its toxin-producing potentialities.

REVIEW OF LITERATURE

Bigelow and Esty (1920) and Esty and Meyer (1922) established that the hydrogen-ion concentration influences the time necessary to destroy spores at a given temperature and, as the pH value is increased within certain limits, the time required for complete destruction is increased. Esty and Meyer (1922) studied this over the pH range 3.5 to 8.8 and found maximum heat resistance of spores in neutral phosphate to be within the pH range 6.3 to 6.9. Bigelow and Cathcart (1921) state that

the lower the pH value of foods, the lower the process necessary for sterilization.

Dickson, Burke, and Ward (1919) concluded that maximum resistance exists when the medium is approximately neutral and rapidly decreases as the hydrogen-ion concentration approaches both acid and alkaline ranges.

Schoenholz, Esty, and Meyer (1933) stated that products with a pH value below 4.5 are not subject to botulinum spoilage when packed under proper sanitary conditions. It was recommended that all products with a pH higher than 4.5 be processed so that all portions of the can are heated sufficiently to destroy the most resistant strain of *Cl. botulinum*.

Stone (1929) reported that *Cl. botulinum* does not grow and multiply in acid media having a pH below 5.4.

Somer and Somer (1932) found that the velocity of thermal destruction varies greatly in different fruit and vegetable juices of the same pH value.

Dack (1943) states that growth of *Cl. botulinum* is uncertain at pH 5.4 or below. Cameron (1938) states that pH 4.5 is the "upper limit" of acid food. In a later report Cameron (1940) reiterates that pH 4.5 is the "dividing line" between acid and nonacid canned foods; he also suggests further subdivisions.

TABLE 1

Classification[1] of Canned Foods With Respect to Their pH Values

Group No.	Group description	pH	Examples of food products
I	Nonacid	7.0 to 5.3	Corn, lima beans, and peas
II	Semi or medium acid	5.3 to 4.5	Beets and pumpkin
III	Acid I	4.5 to 3.7	Apricots, pears and tomatoes
IV	Acid II	3.7 and below	Applesauce, grapefruit, and pickles

[1] Cameron (1940).

Townsend and Powers (1942) point out that extensive tests have shown a pH of 4.5 will inhibit the growth of *Cl. botulinum* in food, and that the California state regulations for control of all acidified vegetable products are based upon this figure.

Certain foods are acidified in commercial practice to a pH below 4.5 in order to inhibit the growth of *Cl. botulinum*. Many of these products are fair or good vehicles for growth of *Cl. botulinum* at their natural pH level, but because these products will not retain good quality when given pressure processes, they are acidified and given boiling-water cooks. Within this classification fall such products as artichokes, broccoli, Brussels sprouts, and potato salad.

NOMENCLATURE

The symbols used in this paper are consistent with those given in papers by Ball (1923, 1928), Townsend, Esty, and Baselt (1938), Olson and Stevens (1939), and others. There are two factors which mathematically describe the thermal death time curves:

F—The number of minutes to destroy a microorganism in a specific medium at 121.1°C.(250°F.).

z—The slope of the thermal death time curve in this medium. It represents the change in temperature necessary to increase or decrease the thermal death time by a multiple of 10.

These symbols which define the thermal death time curve will be used extensively in comparing resistance of bacterial spores in different foods at various pH levels. Conventionally, the subscript zero after F fixes the thermal death time curve slope at 10°C. or 18°F.

EXPERIMENTAL PROCEDURE

Preparation of Stock Suspensions of Test Organisms: All stock spore suspensions of N.C.A. 3679 were prepared as follows: Actively growing cultures of Strain No. 3679 were inoculated into flasks of sterilized liver infusion containing liver particles and iron strips. After an incubation period of 10 days to two weeks, the broth was strained through sterile cheesecloth, then centrifuged. The spores were suspended in a minimum of mother liquor and placed under refrigeration in a screw-cap bottle containing glass beads.

All stock spore suspensions of *Cl. botulinum* type B (N.C.A. Strain No. 213) were prepared as follows: Actively growing cultures were inoculated into flasks containing beef-heart infusion, beef-heart particles, and iron strips. After being incubated for 10 days at 36.7°C.(98°F.), the broth cultures were strained through sterile cheesecloth. The broth was centrifuged, the spores were suspended in a minimum of mother liquor and placed in sterile bottles containing glass beads, and the stock suspensions refrigerated until used.

Standardization of Spore Suspensions: Because of differences in resistances of various spore suspensions, it was necessary to standardize the spore suspensions to be used in inoculated tests. The regular practice of determining the resistance of a spore suspension in standard phosphate buffer solution was followed. This method permits a basis for comparison between inoculation experiments involving different suspensions of

TABLE 2

Resistance of Test Organisms in Standard Phosphate Buffer Solution at 121.1°C.(250°F.)

Test organism	Corrected times [1] at 250°F.		Spore dilution used	Approximate spore count of diluted suspension	Suspension standardized for purée pack
	Survived	Killed			
	min.	*min.*		*per ml.*	
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Asparagus
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Green beans
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Beets
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Beets (repeat)
N.C.A. 3679	3.65	4.15	1-1,000	100,000-150,000	Carrots
N.C.A. 3679	3.15	3.65	1-1,000	150,000	Corn
N.C.A. 3679	4.65	5.15	1-1,000	150,000-200,000	Corn (repeat)
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Peas
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Sweet potatoes
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Pumpkin
N.C.A. 3679	3.65	4.15	1-1,000	150,000	Spinach
Cl. botulinum	6.15[2]	8.15[2]	1-3.5	10,000,000	Corn
Cl. botulinum	8.15[2]	11.15[2]	1-2	10,000,000	Peas
Cl. botulinum	7.15[2]	9.15[2]	1-2	1,000,000	Spinach

[1] Corrections for experimental heating times to compensate for heating lag in the t.d.t. tubes were obtained f om work by Sognefest and Benjamin (1944); 0.85 minute was subtracted from the experimental times to give the corrected times. [2] These times are minutes at 240°F.

organisms. One milliliter of a neutral phosphate dilution was placed in a standard thermal death time tube, and the tube was sealed and heated for various times at 121.1°C.(250°F.). For these tests three to five sealed Pyrex tubes were used for each variable.

PREPARATION OF PURÉES

The raw vegetables were obtained on the local market or from nearby canning plants. These vegetables were cleaned, blanched, and passed through an experimental Fitzpatrick comminuting machine fitted with a No. 1 screen. The finished purée was cooled by being forced through a 3/8-inch diameter, 100-foot length aluminum coil submerged in running cold water, then through a 5/8-inch diameter, 100-foot length coil submerged in crushed ice and brine. The temperature of the purée after cooling varied between 2.8 and 7.2°C.(37 and 45°F.).

The following different products were used for this work: asparagus, green beans, beets, carrots, white and yellow corn, peas, sweet potatoes, spinach, and pumpkins.

The consistency of the purées prepared from these vegetables approximated that of vegetable purées produced commercially. Some water was

TABLE 3

Amounts of Alkali and Acid Added for Adjusting pH of Purées for Thermal Death Time Tests

Type of purée	Normal acid or base per gal.	pH before processing	Type of purée [1]	Normal acid or base per gal.	pH before processing
	ml.			*ml.*	
Asparagus	56.0 HCl	4.49	Corn	131 HCl	4.53
	36.6 HCl	5.00		93 HCl	5.0
	23.3 HCl	5.45		77 HCl	5.5
	0	6.70		39 HCl	6.03
	90.0 NaOH	8.99		20 HCl	6.5
Green beans	27.5 HCl	5.0		0	7.1
	15.35 HCl	5.5		96 NaOH	9.38
	0	6.07	Peas	202.8 HCl[2]	4.51
	20.0 NaOH	7.0		142.8 HCl[2]	5.0
	150.0 NaOH	9.0		88.57 HCl[2]	5.49
Beets	43.3 HCl	4.48		34.66 HCl[2]	6.03
	24.0 HCl	5.0		0	6.66
	0	6.05		11.66 NaOH[2]	7.06
	7.6 NaOH	6.5		33.33 NaOH[2]	8.07
	86.6 NaOH	8.95	Sweet potato	72 HCl	4.53
Carrot	36.6 HCl	4.53		38.4 HCl	4.99
	20.0 HCl	4.99		0	5.61
	0	5.61		35.2 NaOH	7.08
	20.1 NaOH	6.49		73.3 NaOH	8.7
	53.3 NaOH	8.97	Pumpkin	40 HCl	4.52
				21.5 HCl	5.04
				0	5.99
				13.9 NaOH	7.04
				45.3 NaOH	8.99

[1] The amount of acid or alkali added to spinach purées in adjustment of pH was not recorded.
[2] Normal alkali and acid were used for adjusting pH in all packs except for pea purée. For this purée 0.5 N acid and alkali were used.

added during the preparation of these purées and, if additional water was needed, this was added at the time the pH of the batches was adjusted. Adjustment of pH was made by the addition of 1 N HCL or NaOH; pH values were checked by means of a glass electrode.

THERMAL DEATH TIME TESTS IN PURÉES

For all inoculated lots of puréed foods, the 208 x 006 thermal death time can was used. Approximately 14 grams of the cold (45°F.) food material were placed in the cans, and after adding 0.1 milliliter of the properly diluted, standardized spore suspension, the cans were closed under 20 to 25 inches of vacuum, shaken to mix the contents, and transferred to the processing retort.

The processing was done in small retorts especially designed for studying thermal death times. These have been described by Pilcher, *et al.* (1947). Before admitting steam to the retort 20 to 25 inches of vacuum was drawn. Steam was then admitted. By having six of these retorts it was possible to run six tests at once, running each at a different time interval, thereby covering the probable resistance range of the test spores. A total of eight cans was processed for each time studied.

Past work has indicated that although spores may be viable after a heat treatment, the pH may be sufficiently low or the medium sufficiently altered to inhibit growth. Therefore, in order to determine whether or not organisms survived the heat treatment, one sample of the product from each variable was cultured in suitable media immediately following the process. Cans not opened for immediate culture were placed at 98°F. and examined frequently for swells, i.e., gas formation indicative of bacterial growth.

DATA AND RESULTS

The detailed results of thermal death tests made for the purpose of studying the effect of pH on thermal resistance in all products tested are given (Tables 6 to 19.) The heating times given are corrected for retort come-up and heat-penetration lags. The data are condensed and summarized (Table 4).

DISCUSSION

Since the quality of most canned foods may be adversely affected by a severe thermal process, it is desirable to give the canned product the minimum process required to prevent spoilage losses and to eliminate health hazards. It is, therefore, important to be able to estimate within reasonably close limits a suitable process time for a given processing temperature.

Past experience has indicated that processes based upon a spore concentration having a resistance of 3.65 to 4.15 minutes at 250°F. (corrected time) in neutral phosphate, when using test organism N.C.A. No. 3679, should insure a commercially sterile product for nonacid foods. Tanner (1933) defines a commercially sterile canned food as one which has been processed by heat in such a manner that it will not spoil under ordinary market conditions, even though it may not have been completely sterilized.

In calculating an adequate commercial process the only bacteriological data required are the maximum heat resistances (F and z values) of the

TABLE 4

Summary of Thermal Death Time Data

Type of purée	Test organism	Thermal death time of inoculum in phosphate pH 7	pH of product		T.D.T. factors based on can-spoilage results	
			Before heating	After heating at 250°F. for minimum death time	*F*	*z*
Asparagus	N.C.A. 3679	4.15 at 250°F.	4.5	4.6	.....[2]	....[2]
			5.0	5.1	0.16	10
			5.5	5.5	1.10	12
			6.7[1]	6.2	3.60	16
			9.0	8.0	4.30	16
Green bean	N.C.A. 3679	4.15 at 250°F.	5.0	5.1	0.50	13
			5.5	5.5	3.30	22
			6.1[1]	5.9	2.80	18
			7.0	6.2	4.00	17
			9.0	7.5	4.60	17
Beet	N.C.A. 3679	4.15 at 250°F.	4.5	4.8	.....[2]	....
			5.0	5.0	.....[2]	....
			6.1[1]	5.9	3.00	18.5
			6.5	6.2	3.10	18
			9.0	7.1	7.30	20
Beet (repeat)	N.C.A. 3679	4.15 at 250°F.	4.5	4.5	.....[2]	....
			5.0	5.0	.....[2]	....
			5.5	5.5	0.7	10
			6.3[1]	5.8	4.1	18
			6.9	6.2	4.4	18
Carrot	N.C.A. 3679	4.15 at 250°F.	4.5	4.6	.....[2]	....
			5.0	5.0	.....[2]	....
			5.6[1]	5.6	.....[2]	....
			6.5	6.1	.....[2]	....
			9.0	7.9	1.50	16
Corn (white)	N.C.A. 3679	3.65 at 250°F.	4.5	4.7	0.08	16
			5.0	5.2	1.80	18
			5.5	5.7	3.70	17
			6.0	6.1	5.60	20
			6.5	6.6	4.40	17
			7.1[1]	6.9	4.70	17
Corn (yellow)	N.C.A. 3679	5.15 at 250°F.	4.5	4.4	.....[2]	....
			5.0	5.0	0.45	14
			5.5	5.5	3.30	17
			6.3	6.2	7.90	20
			6.8[1]	6.6	9.00	21
Pea	N.C.A. 3679	4.15 at 250°F.	4.5	4.7	0.14	18
			5.0	5.2	1.10	12
			5.5	5.7	5.60	18
			6.0	6.1	5.10	18
			6.6[1]	6.4	5.70	16
			7.0	6.7	6.40	18
			8.0	7.2	6.80	18
Sweet potato	N.C.A. 3679	4.15 at 250°F.	4.5	4.6	.....[2]	....
			5.0	5.0	0.33	11
			5.6[1]	5.5	3.60	16
			7.1	6.1	6.50	22
			8.7	6.9	4.70	20
Pumpkin	N.C.A. 3679	4.15 at 250°F.	4.5	4.6	.....[2]	....
			5.0	5.1	0.15	11
			6.0[1]	6.0	.....[2]	....
			7.0	6.6	.....[2]	....
			9.0	7.6	1.70	14
Spinach	N.C.A. 3679	4.15 at 250°F.	4.5	4.5	.....[2]	....
			4.9	5.0	.....[2]	....
			5.5	5.5	.....[2]	....
			7.0[1]	6.5	.....[2]	....
Corn	*Cl. botulinum*	8.15 at 240°F.[3]	5.5	5.4	0.024	8
			6.9[1]	6.7	1.880	19
Pea	*Cl. botulinum*	11.15 at 240°F.	6.0	6.2	0.64	15
			7.2[1]	7.0	0.72	15
			9.0	8.1	1.50	20
Spinach	*Cl. botulinum*	9.15 at 240°F.	5.0	5.1	.....[2]	....[3]
			5.9	5.7	.....[2]	....[3]
			7.2[1]	7.0	.....[2]	....[3]
			8.6	8.0	0.43	18.5

[1] The natural pH of product before heating. [2] Insufficient data to establish curves. [3] The pH values shown for tests involving this organism were taken after heating at 112.8 or 115.6°C. (235 or 240°F.) for the minimum thermal death time. No 250°F. heating tests were made with *Cl. botulinum*.

types of microorganisms likely to cause spoilage in a given food when placed at ordinary storage temperatures. Information on variable resistances of a strain or the resistance of different species, although of academic interest, is of little value to the technologist charged with the responsibility of establishing commercial processes.

Data given (Table 4) indicate in general that within the pH levels of 4.5 to 9 in a food product before processing, the lower the pH value, the lower the order of process (*F* value) necessary to prevent spoilage.

TABLE 5

Effect on pH Values of Equivalent Processes for a Given Product When Using Test Organism No. 3679 Within Temperature Range 110° to 126.7°C.(230 to 260°F.)

Type of purée	Natural pH before heating	260°F.	250°F.	240°F.	230°F.
Asparagus	6.7	6.4	6.2	6.0	5.6
Green bean	6.1	5.9	5.9	5.7	5.5
Beet	6.1	6.0	5.9	5.7	5.5
Beet	6.3	....	5.8	5.7	5.5
Carrot	5.6	5.6	5.6	5.5	5.5
Corn	7.1	6.9	6.9	6.7	6.5
Corn	6.8	....	6.6	....	6.3
Pea	6.6	6.5	6.4	6.3	6.1
Sweet potato	5.6	5.6	....	5.5	5.4
Pumpkin	6.0	6.1	6.0	5.8	5.7
Spinach	7.0	6.5	6.5	6.0	6.0

In this connection, it is of interest to note that the *F* values for most products which had a pH of 5 were less than 1. The only exceptions were peas and corn. This is very substantially less than the neutral phosphate resistance.

At pH of 5.5 there is a marked rise in *F* values necessary to prevent spoilage. Inconsistent results were obtained within the pH range of 5.5 and 9, but there is a general trend to higher resistances at the higher pH levels. The increase in heat resistance from pH 5.5 to 9 is slight in comparison with the indicated increase from pH 5 to 5.5. Heat resistance in this discussion is defined as the *F* value necessary to prevent spoilage of the product in the can in which it is heated.

Data (Table 4) also indicate there may be no direct correlation between pH and *F* values when comparing one low-acid food product with another. For instance, sweet potato purée with a natural pH of 5.6 before heating and asparagus purée with a pH of 6.7, required an identical *F* value of 3.6 to prevent spoilage, owing to the presence of a standardized spore suspension of N.C.A. No. 3679. Sweet potato and carrot purée had identical natural pH values before heating, but these low-acid vegetables required *F* values of 3.6 and less than 0.35, respectively. Other comparisons show similar results.

The conclusion drawn from these data is that the *F* value required to prevent spoilage varies with the type of product in which the spores of the test organism are heated and incubated after the heat treatment, as well as the pH of the media.

TABLE 6

Spoilage Data for Inoculated Asparagus Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture immediately after process		Subculture of flat cans after incubation		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction	Survival	Destruction	*F*	*z*
°*F.*		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*	*min.*	*min.*		
230	4.5	.....	....	3.35	4.6	28.35	38.35	.....	3.35	.....	....
240		0.02	4.6	0.35	4.6	11.35	.....	0.02	0.35		
250		.....	....	0.02	4.6	3.35	.....	0.16	0.35		
260		0.01	4.6	0.02	4.6	0.85	.....	0.01	0.02		
230	5.0	8.35	5.1	18.35	5.1	48.35	.....	8.35	18.35	0.16	10
240		0.35	5.1	2.35	5.1	11.35	.....	0.35	2.35		
250		0.02	5.1	0.16	5.1	3.35	.....	0.85	1.35		
260		0.01	5.1	0.02	5.1	0.85	.....	0.01	0.02		
230	5.5	48.35	5.3	58.35	5.2	58.35	.....	48.35	58.35	1.1	12
240		6.35	5.5	10.35	5.3	14.35	18.35	6.35	10.35		
250		0.35	5.6	1.85	5.5	6.35	7.85	0.35	1.85		
260		0.02	5.6	0.16	5.5	1.85	2.85	0.02	0.16		
230	6.7	58.35	5.7	78.35	5.6	78.35	.....	.....	58.35	3.6	16
240	(not	10.35	6.1	14.35	6.0	18.35	22.35	.....	10.35		
250	adjust-	3.35	6.2	5.35	6.2	3.35	5.35	.....	3.35		
260	ed)	0.16	6.4	0.35	6.4	1.85	.....	.....	0.16		
230	9.0	58.35	6.9	.....	....	58.35	.....	58.35	.....	4.3	16
240		14.35	7.6	18.35	7.4	26.35	.....	14.35	18.35		
250		2.85	8.1	4.35	8.0	8.85	.....	5.85	7.85		
260		0.35	8.3	0.85	8.2	2.35	3.35	2.35	3.35		

[1] These pH values were taken immediately after heating for indicated time.

TABLE 7

Spoilage Data for Inoculated Green-Bean Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction	*F*	*z*
°*F.*		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	5.0	13.5	5.1	18.5	5.1	13.5	18.5		
240		1.5	5.1	3.5	5.1	3.5	5.5		
250		0.24	5.1	0.5	5.1	0.24	0.5		
260		0.11	5.1	0.22	5.2	0.11	0.22	0.5	13
230	5.5	18.5	5.3	28.5	5.3	28.5[2]	33.5		
240		8.5	8.5	11.5	5.4	5.5	8.5		
250		1.5	5.6	2.5	5.5	6.0[2]	.....		
260		1.0[2]	5.6	.....	....	1.0	.....		
276		.....	....	0.07	....	.....	.....	3.3	22
230	6.1	33.5	5.5	38.5	5.5	28.5	33.5		
240	(not	8.5	5.8	11.5	5.7	2.5	5.5		
250	adjust-	2.5	6.0	3.5	5.9	3.5	6.0		
260	ed	0.55	6.0	1.0	5.9	1.0	.....		
276		.....	....	0.07	....	.....	.....	2.8	18
230	7.0	43.5	5.6	58.5	5.5	38.5	43.5		
240		14.5	5.9	17.5	5.9	21.0[2]	.....		
250		3.5	6.4	4.5	6.2	1.5	2.5		
260		0.55	6.4	1.0	6.4	0.55[2]	.....		
280		.....	....	0.07	....	.....	.....	4.0	17
230	9.0	48.5	6.3	.....	....	18.5	23.5		
240		12.5	6.8	.....	....	6.5	8.5		
250		2.5	7.7	3.5	7.5	2.5[2]	3.5		
260		1.0	7.7	.....	....	0.55	1.0		
274.5		.....	....	0.07	....	.....	.....	4.6	17

[1] These pH values were taken immediately after heating for indicated time. [2] Skips occurred in these series. Cans heated for lesser times gave negative results.

TABLE 8

Spoilage Data for Inoculated Beet Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction		
								F	*z*
°F.		min.	pH	min.	pH	min.	min.		
230	4.5	.....	....	0.02	4.7	18.35	.....		
240		.....	....	0.01	4.6	5.35	.....		
250		.....	....	0.01	4.8	0.85	.....		
260		.....	....	0.01	4.7	0.30	.....	.....	.....
230	5.0	.....	....	18.15	5.1	18.35	24.35		
240		.....	....	1.35	5.0	5.35	7.35		
250		.....	....	0.02	5.0	0.16	1.35		
260		.....	....	0.01	5.1	0.85	.....	.....	.....
230	6.1	23.35	5.6	38.35	5.5	.....	18.35		
240	(not	.....	....	12.35	5.7	.....	12.35		
250	adjust-	2.85	5.9	4.35	5.9	.....	1.35		
260	ed)	0.35	6.1	0.70	6.0	0.02	.15		
283		.....	....	0.07	....	.....	.....	3.0	18.5
230	6.5	28.35	6.0	38.35	5.8	38.35	58.35		
240		11.35	6.1	17.35	5.9	7.35	11.35		
250		2.85	6.3	4.35	6.2	2.85	4.35		
260		0.35	6.5	0.70	6.3	0.40	.70		
287		.....	....	0.07	....	.....	.....	3.1	18.0
230	9.0	58.35	6.4	78.35	6.4	.....	23.35		
240		17.35	6.9	23.35	6.7	7.35	11.35		
250		5.85	7.4	7.35	7.1	1.35	2.85		
260		1.85	7.7	.....	....	.....	0.50		
280		.....	....	0.07	....	.....	.....	7.3	20.0

[1] These pH values were taken immediately after heating for indicated time.

TABLE 9

Spoilage Data for Inoculated Beet Purée[1] Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[2]		Destruction[2]		Survival	Destruction		
								F	*z*
°F.		min.	pH	min.	pH	min.	min.		
230	4.5	.....	....	3.1	4.5	23.1	.....	.....	....
240		.....	....	0.01	4.5	5.1	7.1		
250		.....	....	0.01	4.5	0.65	1.1		
260		.....	....	0.008	4.5	0.01	0.1		
230	5.0	.....	....	8.1	5.0	33.1	.....	.....	....
240		.....	....	1.1	5.0	10.1	.....		
250		.....	....	0.01	5.0	1.1	2.1		
260		.....	....	0.008	5.0	0.06	0.1		
230	5.5	13.1	5.4	18.1	5.4	38.1	.....	0.7	10
240		1.1	5.5	5.1	5.5	14.1	18.1		
250		.....	....	0.1	5.5	2.1	3.1		
260		0.01	5.5	0.06	5.5	1.1[3]	.....		
230	6.3	38.1	5.6	48.1	5.5	48.1	.....	4.1	18
240	(not	11.1	5.8	15.1	5.7	15.1	19.1		
250	adjust-	3.1	5.9	4.1	5.8	4.1	5.1		
260	ed)	1.1[3]	6.0	.....	....	1.1	.....		
230	6.9	48.1	5.8	58.1	5.7	48.1	58.1	4.4	18
240		15.1	6.0	19.1	5.9	15.1	19.1		
250		4.1	6.3	5.1	6.2	4.1	5.1		
260		6.5	6.5	1.1	6.4	1.1	1.6		

[1] This experiment was repeated in order to include additional pH levels to those included in Table 8. [2] These pH values were taken immediately after heating for indicated time. [3] Skips.

TABLE 10

Spoilage Data[1] for Inoculated Carrot Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture	
		Survival[2]		Destruction[2]		Survival	Destruction
°F.		min.	pH	min.	pH	min.	min.
230	4.5	.....	....	8.35	4.6	.....	8.35
240		.....	....	0.35	4.6	3.35	6.35
250		.....	....	0.16	4.6	0.16	0.35
260		.....	....	0.01	4.6	0.01	0.06
230	5.0	.....	....	18.35	5.0	.....	18.35
240		.....	....	3.35	5.0	.....	3.35
250		.....	....	0.16	5.0	0.85	1.85
260		0.02	5.0	0.16	5.0	.....	0.02
230	5.6	.....	....	18.35	5.5	.....	18.35
240	(not	.....	....	7.35	5.5	.....	7.35
250	adjust-	.....	....	0.35	5.6	.....	0.35
260	ed)	.....	....	0.16	5.6	.....	0.16
230	6.5	.....	....	28.35	5.6	28.35	38.35
240		.....	....	7.35	5.9	23.35	29.35
250		.....	....	1.85	6.1	.....	1.85
260		.....	....	0.16	6.3	0.66	0.95
230	9.0	.....	....	38.35	6.5	.....	38.35
240		.....	....	7.35	7.6	.....	7.35
250		1.35	8.1	2.85	7.9	1.35	2.85
260		0.35	8.3	0.85	8.1	0.35	0.85

[1] Because of incomplete survival results the thermal death time curve could not be established with confidence. Hence, the *F* and *z* values are not shown. [2] These pH values were taken immediately after heating for indicated time.

An observation made incidental to this study is that when using approximately equivalent processes with respect to the prevention of spoilage, reduction in the pH value resulting from the heat treatment is a function of the processing temperature in the range of 126.7 to 110°C. (260 to 230°F.), the higher temperature causing the least pH change. Data were obtained showing the effect of equivalent processes within the temperature range 230 to 260°F. on resulting pH values (Table 5). These results would undoubtedly be influenced by the heating lag in the container; in these tests the heating lag was minimized by use of the small container.

In general, the higher the pH of the product before heating, the greater the effect of heat on lowering of the pH value. Within the pH range 5.5 to 6 the pH does not appear to be appreciably affected by the heating times at 230 to 260°F. necessary to achieve sterility in thermal death time cans. For some acidified vegetables within the pH range 5 to 5.5, the pH was 0.2 higher to 0.2 lower after heating to obtain the first negative spoilage result. Most acidified vegetable purées having a pH value of 4.5 before heating had a pH value 0.1 to 0.3 unit higher after heating.

Erratic results were frequently encountered in cultured samples. In some cases survival occurred at the longest times used. This made it impossible to obtain a straight line T.D.T. curve. These data, therefore,

TABLE 11

Spoilage Data for Inoculated White-Corn Purée (Syrup Added) Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 3.65 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction		
								F	*z*
°F.		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	4.5	0.45	4.6	3.35	4.6	13.35	18.35	.08	16
240		0.02	4.6	0.35	4.6	5.35	.....		
250		0.02	4.6	0.16	4.7	0.35	0.85		
260		.....	4.6	0.02	4.6	0.10	0.35		
256		.....	....	0.07	....	.....	.....		
230	5.0	18.35	5.2	33.35	5.2	18.35	33.35	1.8	18
240		5.35	5.2	7.35	5.2	18.35[2]	.....		
250		1.35	5.2	2.85	5.2	0.16	1.35		
260		0.10	5.2	0.35	5.2	0.10	0.35		
267		.....	....	0.07	....	.....	.....		
230	5.5	48.35	5.6	73.35	5.6	18.35	33.35	3.7	17
240		11.35	5.6	17.35	5.6	5.35	11.35		
250		1.35	5.7	2.85	5.7	1.35	2.85		
260		1.35	5.7	1.85	5.7	0.10	0.35		
230	6.0	38.35	6.1	58.35	6.0	38.35	58.35	5.6	20
240		17.35	6.1	23.35	6.1	28.35[2]	35.35		
250		2.85	6.1	4.35	6.1	1.35	2.85		
260		0.70	6.2	1.0	6.2	.....	0.35		
230	6.5	58.35	6.4	78.35	6.3	38.35	58.35	4.4	17
240		12.35	6.5	17.35	6.4	.....	12.35		
250		2.85	6.6	4.35	6.6	1.85	2.85		
260		1.00[2]	6.6	1.25	6.6	0.70	1.00		
230	7.1	58.35	6.5	78.35	6.5	38.35	.....	4.7	17
240	(not	17.35	6.8	23.35	6.7	11.35	17.35		
250	adjust-	2.85	6.9	4.35	6.9	.....	1.85		
260	ed)	1.10	7.0	1.35	6.9	0.85	1.10		
280		.....	....	0.07	....	.....	.....		

[1] These pH values were taken immediately after heating for indicated time. [2] Skips.

could not be analyzed as was the case for the spoilage results obtained in the product packed in T.D.T. cans. This observation is in line with the results obtained by other investigators.

At the natural pH level spores of N.C.A. No. 3679 developed better in pea purée and in corn purée than in the subculture medium.

Tanner (1933) has stated that the protective effect of acidity of foods must be interpreted with considerable care. A food product may have a gross reaction of pH 4.5 or less but still have areas in it where the pH is more nearly neutral, especially if other organisms are growing in it.

SUMMARY

Thermal death time tests were carried out on freshly prepared vegetable purées of asparagus, green beans, beets, carrots, white and yellow corn, peas, sweet potatoes, pumpkin, and spinach, to which various amounts

TABLE 12

Spoilage Data for Inoculated Yellow-Corn Purée (Syrup Not Added) Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 5.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction	*F*	*z*
°*F.*		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	4.5	.....	....	0.50	4.5	16.35	.....		
240		.....	....	0.01	4.4	3.35	5.35	<0.01	....
250		.....	....	0.01	4.4	0.85	1.35		
260		.....	....	0.01	4.4	0.60	0.15		
230	5.0	.....	....	13.35	5.0	23.35	33.35	<0.5	....
240		.....	....	3.35	5.0	7.35	10.35		
250		0.16	5.0	0.50	5.0	0.50	2.85		
260		0.02	5.0	0.50	5.0	0.50	0.85		
230	5.5	43.85	5.5	48.35	5.5	43.35	48.35	3.3	17
240		10.35	5.5	13.35	5.5	18.35	23.35		
250		0.50	5.5	2.85	5.5	0.50	2.85		
260		0.50	5.5	0.85	5.5	0.85	1.35		
230	6.3	68.35	6.1	78.35	6.1	58.35	68.35	7.9	20
240		22.35	6.2	26.35	6.2	26.35	29.35		
250		7.35	6.3	8.85	6.2	7.35	8.85		
260		1.35	6.3	1.85	6.3	0.50	0.90		
230	6.8	68.35	6.4	78.35	6.3	58.35	68.35	9.0	21
240	(Not	29.35[2]	6.4	.....	....	26.35	29.35		
250	adjust-	7.35	6.6	8.85	6.6	5.85	7.85		
260	ed)	2.85[2]	6.6	.....	....	1.35	1.85		

[1] These pH values were taken immediately after heating for indicated time. [2] One can spoiled.

of HCl and NaOH were added in order to modify the natural pH values. Heating tests on a standardized spore suspension of N.C.A. 3679 and, to a smaller extent, spores of *Cl. botulinum,* were made in vegetable purée within the pH range 4.5 to 9.

In order to simulate commercial packing conditions, the vegetable purées were packed in 208 x 006 size cans and processed at various times, at temperatures of 110, 115.6, 121.1, and 126.7°C.(230, 240, 250, and 260°F.).

Resistances of the spore suspensions of No. 3679 used for most of the tests were between 3.65 and 4.15 minutes at 250°F.—the corrected heating time in neutral phosphate. Identical spore concentrations were used for spoilage tests in various vegetables.

Spoilage results indicate that the lower the pH of a raw vegetable within the range 4.5 and 9, the lower the sterilizing value needed to insure a commercially sterile product. The sterilizing value (*F*) for a product with a pH of 4.5 was less than 0.1. At a pH around 5.5 there seems to be a decided rise in the sterilizing value necessary to prevent spoilage. Within the pH range 6 to 9 there seems to be only a slight rise of the sterilizing value with the rise in pH.

The thermal resistance in terms of *F* values, based on can-spoilage results, varied with the different products used in these tests.

Thermal processing of nonacid foods tends to lower the pH. In general,

TABLE 13

Spoilage Data for Inoculated Pea Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before heating	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction		
								F	*z*
°F.		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	4.5	.....	....	3.50	4.6	8.50	18.50	0.1	14.5
240		.....	....	0.50	4.6	1.50	3.85		
250		0.05	4.7	0.22	4.7	0.24	0.50		
260		0.01	4.7	0.05	4.7	0.05	0.24		
268		.....	....	0.07	....	.....	.....		
230	5.0	23.50	5.1	48.50	5.1	23.50	48.50	1.1	12
240		5.50	5.2	11.50	5.2	5.50	11.50		
250		0.24	5.2	1.50	5.2	0.24	1.50		
260		0.05	5.2	0.24	5.2	0.05	0.24		
276		.....	....	0.07	....	.....	.....		
230	5.5	48.50	5.7	78.50	5.6	48.50	78.50	5.6	18
240		17.50	5.6	23.50	5.6	5.50	11.50		
250		3.00	5.7	4.50	5.7	1.50	3.00		
260		1.40[2]	5.7	1.70	5.7	0.65	0.80		
284		.....	....	0.07	....	.....	.....		
230	6.0	48.50	6.0	78.50	5.9	33.50	48.50	5.1	18
240		17.50	6.0	23.50	6.1	17.50	23.50		
250		3.00	6.1	4.50	6.1	.....	3.00		
260		1.10	6.1	1.40	6.1	.....	1.70		
285		.....	....	0.07	....	.....	.....		
230	6.6	78.50	6.1	108.50	6.1	48.50	78.50	5.7	16
240	(not	23.50	6.3	29.50	6.3	17.50	24.50		
250	adjust-	4.50	6.5	6.00	6.4	3.00	4.50		
260	ed)	1.10	6.5	1.40	6.5	0.80	1.10		
285		.....	....	0.07	....	.....	.....		
230	7.0	78.50	6.4	108.50	6.3	48.50	78.50	6.4	18
240		17.50	6.6	23.50	6.6	11.50	17.50		
250		6.00	6.7	7.50	6.7	3.00	4.50		
260		1.40	7.0	1.75	6.9	0.80	1.10		
285		.....	....	0.07	....	.....	.....		
230	8.0	78.50	6.6	108.50	6.5	48.50	78.50	6.8	18
240		17.50	7.0	23.50	6.9	11.50	17.50		
250		4.50	7.4	6.00	7.2	3.00	4.50		
260		1.70	7.5	2.00	7.4	1.10	1.40		
284		.....	....	0.07	....	.....	.....		

[1] These pH values were taken immediately after heating for indicated time. [2] Skips.

the higher the pH of the product before heating the greater the effect of heat in lowering the pH. The higher temperature equivalent processes caused less pH reduction than did the lower temperature processes in the range 260 to 230°F.

In general, cultural results show that spores which did not germinate and spoil products acidified to a pH of 4.5, did germinate when transferred to a more suitable growing medium.

TABLE 14

Spoilage Data for Inoculated Sweet-Potato Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction	*F*	*z*
°*F.*		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	4.5	.....	....	8.35	4.6	8.35	18.35	....	....
240		.....	....	0.35	4.6	3.35	6.35		
250		.....	....	0.16	4.6	0.35	0.85		
260		.....	....	0.01	4.6	0.02	0.16		
230	5.0	18.35	5.0	28.35	5.0	28.35	43.35	0.33	11
240		0.35	5.0	3.35	5.0	6.35	9.35		
250		0.16	5.0	0.35	5.0	1.35	3.35		
260		0.02	5.0	0.16	5.0	0.02	0.16		
230	5.6	38.35	5.5	58.35	5.4	38.35	58.35	3.6	16
240	(not	10.35	5.5	14.35	5.5	14.35	18.35		
250	adjust-	3.35	5.5	.....	....	3.35	.....		
260	ed)	0.35	5.6	0.85	5.6	0.02	0.16		
230	7.1	38.35	5.9	58.35	5.7	28.35	38.35	6.5	22
240		14.35	6.1	18.35	5.6	6.35	10.35		
250		5.85	6.3	7.35	6.1	.....	1.35		
260		1.35	6.5	2.35	6.3	0.16	0.35		
230	8.7	38.35	6.3	58.35	6.1	58.35	.....	4.7	20
240		10.35	6.8	14.35	6.6	18.35	.....		
250		4.35	7.1	.....	....	4.35	.....		
260		0.85	7.5	.....	....	0.85	.....		

[1] These pH values were taken immediately after heating for indicated time.

TABLE 15

Spoilage Data[1] for Inoculated Pumpkin Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before heating	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[2]		Destruction[2]		Survival	Destruction	*F*	*z*
°*F.*		*min.*	*pH*	*min.*	*pH*	*min.*	*min.*		
230	4.5	.....	....	8.35	4.6	8.35	13.35	....	....
240		.....	....	0.35	4.6	1.35	3.35		
250		.....	....	0.16	4.6	0.16	0.35		
260		.....	....	0.01	4.6	0.01	0.06		
230	5.0	.....	....	13.35	5.2	18.35	23.35	0.15	11
240		.....	....	1.35	5.1	3.35	5.35		
250		0.02	5.2	0.16	5.1	0.16	1.35		
260		0.01	5.1	0.02	5.1	0.02	0.16		
230	6.0	.....	....	18.35	5.7	23.35	38.35	....	....
240	(not	.....	....	7.35	5.8	.....	7.35		
250	adjust-	.....	....	1.35	6.0	.....	1.35		
260	ed)	.....	....	0.16	6.0	.....	0.16		
230	7.0	.....	....	18.35	6.2	38.35	58.35	....	....
240		.....	....	7.35	6.5	.....	7.35		
250		.....	....	1.35	6.6	.....	1.35		
260		.....	....	0.16	6.8	.....	0.16		
230	9.0	38.35	6.5	58.35	6.2	.....	23.35	1.7	14
240		.....	....	7.35	7.2	.....	7.35		
250		1.35	7.7	2.85	7.6	.....	1.35		
260		.....	....	0.35	7.9	.....	0.35		

[1] Because of incomplete survival results the thermal death time curve could not be established with confidence. Hence, the *F* and *z* values are not shown for most of this series. [2] These pH values were taken immediately after heating for indicated time.

TABLE 16

Spoilage Data[1] for Inoculated Spinach Purée Packed in T.D.T. Cans, Using Test Organism N.C.A. No. 3679 Having Thermal Death Time of 4.15 Minutes at 250°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture	
		Survival[2]		Destruction[2]		Survival	Destruction
°F.		min.	pH	min.	pH	min.	min.
230	4.5	.....	....	7.10	4.5	11.10	15.10
240		.....	....	1.10	4.5	4.10	5.10
250		.....	....	0.07	4.5	0.07	0.10
260		.....	....	0.009	4.5	0.02	0.06
230	4.9	.....	....	13.10	4.9	23.10	33.10
240		.....	....	3.10	5.0	3.10	5.10
250		.....	....	0.10	5.0	0.30	1.10
260		.009	....	0.10	4.9	0.06	0.30
230	5.5	.....	....	28.10	5.4	.....	28.10
240		.....	....	5.10	5.5	.....	5.10
250		.....	....	0.70	5.5	1.10	2.10
260		.009	5.5	0.60	5.5	0.10	0.65
230	7.0	.....	....	38.10	6.0	88.10	.....
240	(not	.....	....	19.10	6.0	.....	.....
250	adjusted)	.....	....	2.60	6.5	7.10	8.10
260		.....	....	0.60	6.5	2.60	.....

[1] Because of incomplete survival results the thermal death time curve could not be established with confidence. Hence, the *F* and *z* values are not shown. [2] These pH values were taken immediately after heating for indicated time.

TABLE 17

Spoilage Data[1] for Inoculated Corn Purée Packed in T.D.T. Cans, Using Test Organism Cl. botulinum Having Thermal Death Time of 8.15 Minutes at 240°F. in Phosphate pH 7

Temp.	pH before heating	Incubated cans—spoilage results				T.D.T. factors based on spoilage results	
		Survival[2]		Destruction[2]		*F*	*z*
°F.		min.	pH	min.	pH		
230	5.5	5.1	5.4	8.1	5.4	0.024	8
235		0.7	5.5	2.1	5.4		
240		0.3	5.4	2.1	5.4		
230	6.9	20.1	6.6	22.1	6.5	1.88	19
235	(not ad-	10.1	6.6	11.1	6.6		
240	justed)	6.1	6.7	7.1	6.7		

[1] Subculture data not given. [2] These pH values were taken immediately after heating for indicated time.

TABLE 18

Spoilage Data for Inoculated Pea Purée Packed in T.D.T. Cans, Using Test Organism Cl. botulinum Having Thermal Death Time of 11.15 Minutes at 240°F. in Phosphate pH 7

Temp.	pH before process	Incubated cans—spoilage results				Subculture		T.D.T. factors based on spoilage results	
		Survival[1]		Destruction[1]		Survival	Destruction		
°F.		min.	pH	min.	pH	min.	min.	F	z
225	6.0	18.35	6.2	23.35	6.2	13.35	18.35	0.64	16
230		8.35	6.2	13.35	6.2	13.35	18.35		
235		4.35	6.2	6.35	6.2	.....	.....		
225	7.2	28.35	6.8	33.35	6.8	18.35	28.35	0.72	15
230	(not adjusted)	13.35	6.9	18.35	6.8	13.35	18.35		
235		6.35	7.0	8.35	7.0	6.35	8.35		
225	9.0	23.35	7.9	28.35	7.7	.....	.....	1.50	20
230		13.35	8.0	18.35	7.9	.....	.....		
235		6.35	8.2	8.35	8.1	.....	.....		

[1] These pH values were taken immediately after heating for the indicated time.

TABLE 19

Spoilage Data[1] for Inoculated Spinach Purée Packed in T.D.T. Cans, Using Test Organism Cl. botulinum Having Thermal Death Time of 9.15 Minutes at 240°F. in Phosphate pH 7

Temp.	pH before heating	Incubated cans—spoilage results				Subculture	
		Survival[2]		Destruction[2]		Survival	Destruction
°F.		min.	pH	min.	pH	min.	min.
222.5	5.0	.....	....	1.10	5.1	.....	.....
230.0		.....	....	0.30	5.2	.....	.....
237.5		0.01	5.1	0.06	5.1	.....	.....
222.5	5.9	10.10	5.8	13.10	5.7	13.10	.....
230.0		4.10	5.8	.....	....	4.10	.....
237.5		0.65	6.0	.....	....	0.65	.....
230.0	7.2	.....	....	14.10	6.7	.....	.....
237.5	(not adjusted)	.....	....	4.10	7.0	.....	.....
245.0		.....	....	0.30	7.1	.....	.....
230.0	8.6	14.10	7.4	17.10	7.4	.....	.....
237.5		.....	....	4.10	7.9	.....	.....
245.0		0.30	8.2	1.10	8.2	.....	.....

[1] Because of incomplete survival results the thermal death time curve could not be established with confidence. Hence, the *F* and *z* values are not shown. [2] These pH values were taken immediately after heating for the indicated time.

ACKNOWLEDGMENT

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PASTEURIZATION OF BLACK WALNUT MEATS[1]

HARVEY D. ERICKSON[2] AND ARTHUR R. COLMER[3]
Division of Forestry and Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia

[Received for publication, November 21, 1947]

In those areas of West Virginia where black walnut trees *(Juglans nigra L.)* are found, many families supplement their income by harvesting and selling the nuts. Although a great weight of these nuts is sold in the husk, an appreciable poundage of them is shelled in the home and the prepared meats sold to dealers. Some state agencies have devised programs to increase this type of supplementary income, and there is a likelihood of an increase in the production of shelled black walnuts from this source. Besides the meats secured from home shelling, buyers have installed machinery for the large-scale operations attendant upon cracking the nuts and processing the meats. It is possible, therefore, for the quantity and quality of the meats handled by any dealer to be subject to the variation inherent in this dual mode of securing black walnut meats.

Macroscopic examination of random samplings of black walnut meats discloses great variation in the amount of visible foreign material present, and bacteriological examinations show wide variation in the microbial content.

Ostrolenk (1942) and Tanner (1944) have reviewed briefly the microbiology of nut meats. Ostrolenk and Welch (1941) and the Association of Official Agricultural Chemists (1942) have suggested methods for the microbiological examination of nut meats and nut products. Wenzel and Black (1939) found bacterial counts to range from 1,562 to 107,000 per gram of black walnut meats when they are plated on dextrose agar and incubated aerobically at 37°C.(98.6°F.) for 24 to 48 hours. The predominating types of bacteria appeared to be members of the genus *Bacillus*, with some cocci present. The yeast and mold counts ranged from 10 to 1,820 per gram of meats. Ostrolenk and Hunter (1939) noted that 45 per cent of the black walnuts from retail market samples gave positive tests for *Escherichia coli* in decimal dilutions representing 0.01-gram portions. In domestically shelled samples of various nut meats the highest incidence of *E. coli* (68 per cent) was found in black walnuts, which present the greatest problem in shelling and control of sanitary conditions from tree to packaging. Artificially contaminated nut meats stored at room temperaure contained viable test bacteria *(E. coli)* for approximately 68 days.

The growth of black walnut trees in pastures and wood lots, where animals are likely to be present, increases the possibility of coliform contamination of the nuts; this, together with the production of the shelled

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meats under family and dealer conditions where varied sanitary facilities exist makes the meats particularly liable to contamination with organisms of intestinal origin. The increasing control of interstate shipment of black walnut meats by regulatory agencies makes it imperative that ways be found to meet their requirements pertaining to this foodstuff. This study was initiated with a view to finding a satisfactory pasteurization procedure which would insure the proper control of the coliform content of these nut meats but at the same time would not impair the characteristic qualities which make them salable.

MATERIALS AND METHODS

Cultures: Since *E. coli* serves as the indicator organism of intestinal pollution, it was chosen for the studies on pasteurization. Three representative cultures of *E. coli* were used. One was obtained from the American Type Culture Collection as No. 26, another was from the University departmental culture collection, and the third was freshly isolated from feces. All cultures had the IMViC relation of + + − −. One departmental stock culture of *Aerobacter aerogenes* was used.

Media: Fermentation tubes of lactose broth conforming to the requirements of the American Public Health Association (1936) were used in all primary seeding of the meats or dilution waters. Bacto brilliant green bile two-per cent broth was used as the liquid confirmatory medium, and bacto Levine eosin methylene blue agar as the solid confirmatory medium.

Nut Meats: The black walnut meats were secured from a commercial dealer and were comparable with meats offered on the market.

Preparation of Inoculated Meats: Nut meats free from fine particles were used in nearly all tests. "Fines" were removed with a ¼-inch mesh screen. It was found that the presence of these fine particles made inoculation of the meats difficult owing to the "mush" which was formed when the cell suspension of the test organism was added. The test organisms were grown on tryptone glucose extract agar slants at 98.6°F.(37°C.) for 48 hours. The total number of organisms available for a test run could be varied by the use of different numbers of slants. After incubation the organisms were washed from the slants with sterile, distilled water. This suspension was made to a known volume of liquid (usually 100 to 150 ml.), then agitated in a sterile Waring blendor for one minute in an attempt to secure a uniform suspension. A known weight of meats (usually 100 to 150 grams) in a sterile flask was covered with the cell suspension and the mass was agitated intermittently during a 15-minute soaking period. The meats were drained five minutes and finally dried for three hours in an incubator at 98.6°F. A later modification of the drying procedure was used in which the meats were dried three hours at 98.6°F., followed by further drying at room temperature for approximately 20 hours.

Kiln Used for Pasteurization: The desired conditions of temperature and humidity were obtained by using a small experimental dry kiln with forced-air circulation. The insulated cabinet was electrically heated. The humidity was obtained by allowing hot water to spray on recurved folds of hardware cloth in a special unit of the cabinet. Temperature and relative humidity were automatically controlled by thermoregulators, relays, and a solenoid valve. The fluctuation in wet-bulb readings during normal

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Method of Determining Moisture Content of Nut Meats: Two methods were tried for determining the moisture content of the nut meats. Since the oils of nut meats are volatile to some extent, it seemed the most accurate method would be to dry the meats to constant weight in a desiccator over concentrated H_2SO_4. This method was tried on samples from two different pasteurization runs, but its slowness made it unsatisfactory. Consequently, the method of oven-drying the nut meats at a temperature of 101.7°C.(215°F.) was used as the basis for measuring moisture content. The period of oven-drying was established at 19 to 20 hours. After the meats had been dried in the oven, they were allowed to cool in a desiccator, then weighed. The results are expressed as per cent moisture content.

Preparation of Meats for Organoleptic Tests: In one attempt to determine the effect of pasteurization on flavor and texture, meats from the same batch were divided into two portions. One portion was pasteurized at 71.1°C.(160°F.) and 80 per cent relative humidity for 30 minutes; the other portion was left untreated. Glass containers of 250-ml. capacity were filled with the pasteurized meats, then closed with screw-type closures; the unpasteurized meats were treated similarly. Three storage temperatures were used: 3.3 to 4.4°C.(38 to 40°F.), 18.3 to 26.7°C.(65 to 80°F.), and 40°C.(104°F.). The last-named temperature was chosen to serve as an accelerated test. After varying times of storage the meats were examined by tasters who judged their flavor and texture and who then attempted to place them in order of freedom from defects.

In the second attempt to determine if flavor and texture were altered by pasteurization, the same procedure was carried out on another batch of nut meats, except that the storage at 104°F. was omitted.

Before making a taste test the meats from the cold room were spread out and exposed to room conditions for one day in order to make their moisture content more comparable with meats stored at room temperature.

The effect of temperatures above 160°F. on flavor and texture was the object of the third and less intensive taste test. One batch was treated at 82.2°C.(180°F.) and 70 per cent relative humidity for 30 minutes. A second was treated at 93.3°C.(200°F.) and 20 per cent relative humidity for 20 minutes. A third batch was heated in an electric drying oven at 148.9°C.(300°F.) for 10 minutes. One-half of each treated batch and an untreated control sample were stored at room temperature, while the other portions were stored at 4.4°C.(40°F.).

RESULTS

Results at 150°F. With 80 and 90 Per Cent Relative Humidity: The choice of 65.6°C.(150°F.) and 80 per cent relative humidity as starting points for pasteurization trials was an arbitrary one. The results of several experimental runs made under this first pair of kiln conditions are given (Table 1). These conditions did not adequately reduce the numbers of *E. coli.* Also shown are the results of typical tests when the temperature of the kiln was kept at 150°F., but the relative humidity was raised to 90 per cent. These trials showed that increased relative humidity increased the percentage of organisms killed in a given time of treatment and permitted shorter times of treatment; however, texture of the meats was

TABLE 1

Most Probable Number of Escherichia coli per Gram of Nut Meats

Sampling interval	Treatment at 150°F. and 80% relative humidity			Treatment at 150°F. and 90% relative humidity		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3[1]
min.						
0	933,000	150,000	2,880,000	10,100	10,000	860,000
5	.....	.....	.....	9.3	4.3	1.9
10	.....	.....	399.0	3.2	2.3	0.32
15	428.0	702.0	.....	2.7	2.3	1.1
20	.....	.....	150.0	1.2	1.0	0.0
25	.....	.....	.....	.....	.....	.....
30	62.2	130.0	133.0	.....	.....	.....
35	.....	.....	.....	.....	.....	.....
40	.....	.....	27.5	.....	.....	.....

[1] On "fines" (meats passing through ¼-inch mesh screen).

impaired, and difficulties were encountered in maintaining this high humidity. The departmental stock culture of *E. coli* was used in all tests. The initial moisture content of the meats ranged from nine to 12 per cent.

Results at 160°F. and 60, 70, and 80 Per Cent Relative Humidity: Some of the results when the temperature was raised to 160°F. and the relative humidity was lowered to 80 per cent (Tables 2 and 3) show that the use of these conditions was successful for accomplishing killing without undue quality deterioration.

TABLE 2

MPN of E. coli per Gram of Nut Meats at 160°F. and 80 Per Cent Relative Humidity[1]

Sampling interval	Test 1	Test 2	Test 3	Test 4[2]	Test 5[3]	Test 6[4]
min.						
0	7,420	410,000	193,000	40,000	792,000	19,700,000
5	.....	.....	24.0	3.3	.....	3.0
10	2.3	2.3	2.3	0.6	2.0	3.3
15	.....	.....	1.5	0.2	.....	2.6
20	0.8	1.2	1.6	0.4	2.3	2.3
25	.....	.....	.....	.....	.....	.....
30	1.1	0.4	.....	.....	0.3	.....
35	.....	.....	.....	.....	.....	.....
40	0.0	0.0	.....	.....	0.0	.....

[1] Stock *E. coli* was used on Tests 1 to 5, inclusive. [2] Inoculated meats stored 10 days before heat treatment. [3] On "fines" (meats passing through ¼-inch mesh screen). [4] Stock *A. aerogenes* on "fine-free" meats.

The MPN of coliforms present per gram of meats when the laboratory stock cultures of *E. coli* and *A. aerogenes* were used are shown (Table 2). Test 4 was made on meats which had been regularly inoculated and dried. At that time the MPN was 493,000 per gram. These meats were held at 10°C.(50°F.) for 10 days before being pasteurized, during which time the MPN was reduced to 40,000 per gram.

Results obtained when *E. coli* ATCC No. 26 and the *E. coli* freshly isolated from feces were used are recorded (Table 3). Test 4 (Table 3) shows the findings when the nut meats were preheated to the temperature

of the kiln, but at the same time protected from the moisture of the kiln. The original number recorded (Table 3) is the MPN of the unheated meats; the MPN of the meats at the end of the 14 minutes of preheating was 2,920; and an MPN of 0.6 was obtained at the end of 30 minutes' exposure to the conditions of the kiln without any intervening samples being taken.

Results when the nut meats were put on a rubber apron several folds thick, so that they were in a mass approximately eight inches long, six inches wide, and one inch thick, are enumerated in Test 8 (Table 3). Thus, circulation of the heated, moist air through the mass of meats was impaired. At the end of the 30-minute heating period, one sample was taken from the outer layer of the mass and one from the protected interior. A slightly higher bacterial content was obtained from the meats of the interior as compared with those from the surface layer; however, the degree of killing occurring in the interior was good when one considers the thick layer of nut meats used.

Inasmuch as 80 per cent relative humidity at 160°F. seemed to give effective reduction in numbers of *E. coli*, it was thought possible that lower humidities at this same temperature might also be satisfactory in the destruction of *E. coli*. Results when the temperature was held at 160°F., but relative humidities were dropped to 70 and then 60 per cent (Table 4) indicate the conclusion that treatment at 160°F. and 60 per cent relative humidity for 30 minutes did not consistently reduce the *E. coli* to a satisfactory level. Treatment at 160°F. and 70 per cent relative humidity for 30 minutes markedly reduced these organisms in the first four tests. The initial moisture contents of the nut meats in these tests were above 12 per cent.

Since the original mode of drying the nut meats following inoculation caused a higher initial moisture content than would be encountered in some meats ready to be pasteurized under commercial conditions (a range of nine to 12 per cent moisture was usually present in tests shown in Tables 2 and 3), it was thought necessary to investigate the effect of the initial moisture content of nut meats upon the reduction of bacterial numbers, using a pasteurization condition which previous tests had shown to be effective in killing and which, at the same time, should be easy to obtain commercially. Consequently, nut meats with lower initial moisture content were used in succeeding tests.

Although no clear-cut conclusion can be drawn from the data concerning the effect of initial moisture content of the nut meats on the reduction of coliforms during the heat treatment, the data (Table 4) show that pasteurization at 160°F. and 70 per cent relative humidity for 30 minutes produced satisfactory killing of *E. coli* when the moisture content was in the range of 12 to 21 per cent. When the initial moisture content of the meats was seven per cent or less, the above treatment was not satisfactory. Apparently, in cases such as this where the particular combination of temperature and relative humidity is effective on moist nut meats but not on dry meats, it is necessary to resort to higher temperature or higher relative humidity in pasteurizing dry meats.

TABLE 3

MPN of E. coli per Gram of Nut Meats at 160°F. and 80 Per Cent Relative Humidity[1]

Sampling interval	Test 1	Test 2	Test 3	Test 4[2]	Test 5 (7.2% m.c.)	Test 6[3]	Test 7	Test 8	
min.				147,000				Exterior	Interior
0	3,290,000	2,750,000	9,180,000	2,920	42,800,000	6,220,000	3,990,000	180,000	
5	1,970	792.0	10.1	.....	.....	.....	474.0	.....	
10	.....	147.0	62.0	.....	.....	240.0	6.7	.....	
15	73	240.0	.....	.....	.....	.....	.....	.....	
20	27	32.9	6.2	.....	.....	70.2	6.2	.....	
25	.....	.....	.....	.....	.....	.....	.....	.....	
30	.....	.....	0.3	0.6	2.3	17.0	7.3	6.2	0.2

[1] ATCC No. 26 *E. coli* was used on "fine-free" meats in Tests 1 to 5, inclusive. [2] Kiln not opened—preheated. [3] *E. coli* from feces was used in Tests 6 to 8 on "fine-free" meats.

TABLE 4

MPN of E. coli per Gram of Nut Meats[1]

Sampling interval	Treatment at 160°F. and 60% relative humidity						
	Test 1 (20.9% m.c.)	Test 2 (20.9% m.c.)	Test 3 (5.6% m.c.)	Test 4	Test 5		
min.							
0	19,300,000	7,920,000	792,000	2,210,000	9,330,000		
30	33.4	4.5	>2,300[2]	23.1	56.0		
	Treatment at 160°F. and 70% relative humidity						
	Test 1 (20.9% m.c.)	Test 2 (20.9% m.c.)	Test 3 (12.5% m.c.)	Test 4 (12.5% m.c.)	Test 5 (5.6% m.c.)	Test 6 (7.2% m.c.)	Test 7 (7.2% m.c.)
0	19,300,000	7,920,000	9,180,000	28,800,000	792,000	>230,000,000	42,800,000
10	.....	.....	169.0	622.0	.....	.....	.....
20	.....	.....	3.2	11.0	.....	.....	.....
30	1.1	0.2	0.4	0.5	42.8	>1,300	47.4

[1] ATCC No. 26 *E. coli* was used. [2] M.c. denotes moisture content before treating. [3] > denotes that dilution series used failed to give total count; numbers shown are at least minimal values.

Results at Temperatures Above 160°F. With Various Relative Humidities: It is difficult to operate constant temperature kilns at relative humidities approaching saturation because a few degrees lower temperature of any part of the interior surface will bring the moist air to the dew point and result in condensation. To maintain constant humidity, water vapor must be supplied to replace losses by condensation and by vapor leaks. At lower relative humidities, the difference between the air temperature and the dew point is greater, and also, any ordinary variation in the control of wet-bulb temperatures has less effect on the percentage of relative humidity. It is also true that nut meats exposed to air with a high relative humidity will have a higher moisture content than meats exposed to air of lower relative humidity. Therefore, if the moisture content of the meats after pasteurization is of concern from the standpoint of texture and preventing mold growth during storage, one can dry the meats if they are too moist after they leave the pasteurizer, or one can use a pasteurizing process which will leave the nut meats with a low moisture content at the end of the treatment. These considerations led to the tests using relative humidities below 80 per cent and compensating with temperatures above 160°F.

Pasteurization at 170°F. and 60 per cent relative humidity did not adequately reduce the coliform count (Table 5). In the next set of tests the temperature was held at 170°F., but the relative humidity was raised to 70 per cent. This combination gave a fair kill of bacteria but was not considered satisfactory because of the rather high number that remained viable in Test 3. For pasteurization at 70 per cent relative humidity to be effective on dry meats, temperatures higher than 170°F. should be used. The third section (Table 5) shows that a treatment for 30 minutes at 180°F. and 70 per cent relative humidity was satisfactory for the destruction of bacteria on meats of low initial moisture.

At a temperature of 200°F. and a relative humidity of 20 per cent, a satisfactory kill of *E. coli* was not obtained. The humidity was too low and more than offset the 20°F. increase in temperature over the previous set of tests. In the other tests at 200°F. the relative humidity was raised to 50 per cent. Excellent destruction of bacteria was obtained not only for a treating period of 30 minutes but also for periods of 15 and 20 minutes (Table 5).

Two tests were made at 300°F. The nut meats were spread on paper in a thin layer and placed in an electric oven for 10 minutes. Aside from the fact that the meats were altered in flavor and texture, it appears that a longer heating period is necessary to give adequate and dependable killing of *E. coli.*

Moisture Determination: The moisture content of the nut meats after pasteurization varied considerably depending upon the initial moisture content, the humidity and temperature during treatment, the time of treatment, preheating of the nut meats, the thickness of the layer of meats, and the extent of drying taking place after the nut meats were removed from the pasteurizer. When nut meats at room temperature were placed directly into the pasteurizer, moisture was condensed on their surfaces. Hence, the moisture content usually was higher by two to four per cent

TABLE 5

MPN of E. coli per Gram of Nut Meats With Various Treatments[1]

Sampling interval	Treatment at 170°F. and 60% relative humidity		Treatment at 170°F. and 70% relative humidity				Treatment at 180°F. and 70% relative humidity		
	Test 1 (7.3% m.c.)[2]	Test 2 (7.3% m.c.)	Test 1 (7.3% m.c.)	Test 2 (7.3% m.c.)	Test 3 (5.5% m.c.)	Test 4 (5.1% m.c.)	Test 1 (5.2% m.c.)	Test 2 (5.6% m.c.)	Test 3 (4.9% m.c.)
min.									
0	197,000,000	>2,300,000[3]	197,000,000	>2,300,000	15,000,000	10,700	54,200,000	1,690,000	1,160,000
30	561	275	4.3	2.8	62.2	1.3	0.5	0.8	0.3

	Treatment at 200°F., 20%	Treatment at 200°F. and 50% relative humidity						Treatment at 300°F., dry heat only		
	Test 1 (7.3% m.c.)	Test 1 (5.5% m.c.)	Test 2 (5.1% m.c.)	Test 3 (5.1% m.c.)	Test 4 (5.2% m.c.)	Test 5 (5.6% m.c.)	Test 6 (4.9% m.c.)	Test 1 (5.5% m.c.)	Test 2 (5.1% m.c.)	Test 3 (5.2% m.c.)
0	>2,300,000	15,000,000	10,700	4,280	54,200,000	1,690,000	1,160,000	15,000,000	10,700	54,200,000
10	.....	.....	.....	.....	.....	.....	.....	27.5	1.1	19.3
15	.....	.....	.....	0	.....	.....	0	.....	.....	.....
20	>2,300	.....	.....	.....	.....	0	.....	.....	.....	.....
30	.....	0.3	1.1	0	0	.....	.....	.....	.....	.....

[1] ATCC No. 26 *E. coli* was used. [2] M.c. denotes moisture content before treating. [3] > denotes that dilution series used failed to give total count; numbers shown are at least minimal values.

TABLE 6

Moisture[1] of Nut Meats at Various Intervals of Exposure to Kiln Conditions

Sampling interval	150°F. and 60% r.h.[2]	150°F. and 80% r.h.			150°F. and 90% r.h.			160°F. and 60% r.h.			160°F. and 70% r.h.					
	Test 1	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3[3]	Test 4	Test 5	Test 6
min.																
0	3.7	9.8	9.6	9.6	11.5	9.8	3.5	3.8	20.9	5.6	12.5	3.8	3.8	20.9	5.5	7.2
5	.....	.....	.....	.....	.....	.....	5.7	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	.....	.....	.....	.....	15.6	12.8	5.6	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	.....	10.6	10.4	10.5	.....	.....	5.8	.....	.....	.....	.....	.....	.....	.....	.....	.....
20	.....	.....	.....	.....	13.4	10.3	6.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	4.2	9.5	9.3	9.6	.....	.....	.....	4.1	17.4	5.4	9.9	4.7	4.0	17.2	6.0	6.6
40	4.3	.....	.....	8.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

Sampling interval	160°F. and 80% r.h.			170°F., 60% r.h.	170°F. and 70% r.h.			180°F. and 70% r.h.			200°F., 20% r.h.	200°F. and 50% r.h.					300°F., dry heat	
	Test 1	Test 2	Test 3	Test 1	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 1	Test 2	Test 3	Test 4	Test 5	Test 1	Test 2
min.																		
0	5.3	3.3	7.2	7.3	7.3	5.5	5.1	5.2	5.6	4.9	4.0	5.5	5.1	5.2	5.6	4.9	5.5	5.1
5	7.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.0	1.7
15	6.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.4	.....	.....	.....	.....	.....
20	6.4	5.3	.....	.....	.....	.....	.....	.....	.....	.....	2.7	.....	.....	.....	.....	.....	.....	.....
25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	.....	.....	7.9	6.9	6.9	5.5	5.5	5.2	5.6	5.3	.....	4.5	4.0	4.2	5.0	4.8	.....	.....
40	.....	4.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[1] Expressed in per cent. [2] R.h. denotes relative humidity. [3] Meats were preheated in weighing bottle in kiln for 10 minutes, then spread on the tray.

at the five- or 10-minute sampling period than either before or after these intervals. In a process of pasteurization, these variables must be considered. The changes in per cent of moisture of meats with different initial moisture content and subjected to varied conditions of exposure are shown (Table 6).

From the standpoint of ease of operation and economy in pasteurizing, it is desirable to package the meats as they come from the pasteurizer. To do this successfully one must employ that temperature and humidity combination which, while killing the organisms, will add little or no moisture to the nut meats by the end of the treating time.

If nut meats with a high moisture content are taken from the pasteurizer and bottled in non-sterile bottles, it is quite likely that molds will grow on them. At a moisture content of 10 per cent, molds usually become visible in a few days. One sample with 6.4 per cent moisture content, held at room temperature, developed some mold growth in three weeks in the bottom part of a sealed bottle. Two samples, one at 6.0 per cent moisture content and the other at 4.9 per cent, did not have mold growth after 24 days at room temperature. From these results, it is recommended that for preventing mold growth without detriment to the texture the moisture content of the nut meats be about five per cent.

Scoring of Nut Meats: A few of the tasters could not distinguish between any degree of rancidity and the normal taste of fresh black walnuts; these tasters were removed from the taste panel. The results of storage tests on two different lots of meats treated at 160°F. and 80 per cent relative humidity for 30 minutes are given (Table 7). The numbers

TABLE 7

Relative Rank in Quality of Nut Meats Treated at 160°F. and 80 Per Cent Relative Humidity, After Various Periods and Conditions of Storage

Storage period	Number of tasters	Cold room		Room temperature		Oven at 40°C. (104°F.)	
		Pasteurized	Unpasteurized	Pasteurized	Unpasteurized	Pasteurized	Unpasteurized
wk.							
Lot 1							
0	4	.....[1]	.....[1]	.....[1]	.....[1]	.....[1]	.....[1]
4	3	1.0	1.6	1.8	1.6	3.0	3.6
7	4	1.4	1.3	1.0	1.4	3.1	3.1
42	5	1.0	1.0	.....	.....	.....	.....
Lot 2							
0	8	.....	.....	1.4	1.0		
2½	3	.....	.....	1.0	1.0		
6	6	1.0	1.0	1.5	1.8		
15	9	1.0	1.0	2.0	1.9		
21	7	1.0	2.4	2.9	3.3		

[1] No difference between pasteurized and unpasteurized.

in the table are the ranks of the samples judged on the basis of flavor and texture. Each sample in the group for a certain storage period was ranked by each taster so that the best sample had a value of one, the next best a value of two, etc. If two samples were rated equally by the taster, equal value was given. The relative rank of each sample as given in the table

is the ratio of the total score of each sample to the total score of the best sample in the particular group. These relative ranks are directly comparable only with those samples belonging to the same group of tests.

There was little or no difference in flavor between pasteurized and unpasteurized meats 24 hours after the heat treatment when the portions were bottled. In Lot 2 several tasters favored the unpasteurized meats, but after two and one-half weeks of storage they found no difference in taste. In general, there was little difference between the rating of pasteurized and unpasteurized meats for a given storage temperature and storage period. There was, however, a difference in the quality of meats, both pasteurized and unpasteurized, caused by storage temperature and length of storage. In Lot 1 the meats stored at 104°F. were quite rancid after four weeks; whereas, those stored either at room temperature or in the cold room were of good flavor. The same was true at seven weeks. Although in Lot 2 the meats stored at room temperature for 15 weeks were lower in rank than those stored in the cold room, the lower ranking samples were still of fair quality. At the end of 21 weeks, however, the flavor in the meats stored at room temperature was definitely inferior.

Pasteurization temperatures up to 200°F. do not seriously destroy the black walnut flavor. In the upper range, a slightly milder and less bitter flavor seems to result. On the other hand, heating the nut meats at 300°F. for 10 minutes almost completely destroys the typical black walnut flavor, and the dominant flavor is that of roasted nut meats. This temperature also gives the meats a browned appearance and a very dry texture. Trial tests of the effect on flavor of storage at room temperature indicate that treatment of the meats at 180°F. and 70 per cent relative humidity does not impair keeping qualities. No taste tests were made on meats treated at 200°F. and 50 per cent relative humidity, but a sample treated at 200°F. and 20 per cent relative humidity did not maintain flavor quite as well as meats treated at lower temperatures. Nut meats treated at 300°F. had the poorest keeping qualities. Rancidity developed in two to three weeks. As was found in other taste tests, the samples kept in cold storage were much slower in developing rancidity. Additional taste tests are needed for meats treated at the higher temperatures and at various humidities.

DISCUSSION

Samples of black walnut meats as received from dealers varied in their MPN of *E. coli* per gram. One sample had an MPN of 0.78, a second had 680, and a third had 1,230 per gram of meats. The experimental inoculations were made purposely high so that some idea could be gained concerning the temperature, relative humidity, and time interrelationship necessary to reduce a heavy load of coliforms; yet some inoculations were made so as to secure numbers comparable with those found on commercial meats.

Pasteurization of nut meats must first kill pathogenic microorganisms, and at the same time not change the texture and the flavor of the meats to the extent that they lose their salable properties. Any operator of a pasteurizer who attempts to achieve the above objectives must choose wisely those interrelated conditions of pasteurization which will yield those objectives. If, for example, a high relative humidity is used, such as 150°F.

and 90 per cent relative humidity (Table 1), killing of *E. coli* may be accomplished, but the texture of the treated meats will be harmed. On the other hand, when a relative humidity of 60 per cent was used even at a temperature of 170°F., the texture was not harmed as much, but the *E. coli* content was not satisfactorily reduced (Table 5). Similarly, a temperature of 200°F. and a relative humidity of 20 per cent gave dry meats at the end of the heat treatment but failed to give adequate killing.

From the standpoint of ease of control, it is generally true that high relative humidities are more difficult to control than low ones. It is thought that a relative humidity of 70 per cent or less presents the least difficulty in control. Also, from the standpoint of final moisture content and its relation to keeping qualities of the meats, the use of a relative humidity of 70 per cent is to be recommended. On the other hand, when one considers the effect on texture of the final moisture content of the meats, the lower limit of the relative humidity used should be about 50 per cent. Therefore, it appears that to secure both good keeping qualities and satisfactory texture a relative humidity within the range of 50 to 70 per cent should be used in the pasteurizer.

It was found also, during the course of the experiment, that not only was the relative humidity in the pasteurizer of marked importance as a factor affecting the destruction of microorganisms but the moisture content of the meats themselves prior to heat treatment was of significance. Too, whenever heat is used as a lethal agent on microorganisms, the time of its action is an important factor. The tests show that the greatest percentage of kill of the original inoculated microorganisms occurred during the first five minutes of pasteurization; after this period, the rate of kill diminished. That the length of time of pasteurization needed to destroy *E. coli* may be less than the usual 30 minutes when 200°F. and 50 per cent relative humidity is used is illustrated (Table 5). It seems probable that the time needed for proper pasteurization may be varied only if all of the interrelated factors concerned in the process are in balance.

It is difficult to assess the effect on the destruction of *E. coli*, both of preheating the meats and of varying the thickness of the layer of meats in the kiln. Any pasteurizer designed with a hopper to feed meats onto a conveyor must consider these two contingencies. In the case of the former, one test showed a reduction in numbers of *E. coli* owing to this initial heat, and it was observed that there was less moisture condensed on the nut meats after preheating than was usually the case with the other tests. It is thought that better results will be obtained from the heat treatment if the meats are adequately exposed to the heated, moist air.

Pasteurization at 160°F. and 80 per cent relative humidity had no deleterious effect upon the flavor of black walnuts; whereas, pasteurization at 200°F. and 50 per cent relative humidity slightly altered the taste by eliminating some of the pungent flavor usually present in these nut meats, thereby rendering them somewhat milder. Pasteurization with dry air at 300°F. markedly destroyed the typical black walnut flavor and introduced a roasted flavor. The problem of detecting alterations in flavor of foodstuffs is a difficult one at best; and when black walnut meats are the test food, the problem becomes more difficult since the characteristic flavor

of the meats tends to mask the changes. Dustman (1936), Wright (1941), and Brison (1945) have noted the inability of chemical tests to serve as an indicator of rancidity in nut meats. Crist and Seaton (1941) found that organoleptic tests were not entirely satisfactory with the foodstuffs they were testing. The findings of this study are in keeping with the observations of these workers.

A few of the questions on the subject of pasteurization of black walnut meats have been answered by this study. The work was limited in its scope because circumstances prevented the study of other phases of the problem as originally contemplated. The effects of other possible combinations of time, temperature, and relative humidity used in the kiln, together with the concomitant effects introduced by the inoculated meats on the killing of these microorganisms and on the flavor, texture, and keeping qualities of black walnut meats, are suggested for further study.

SUMMARY

Combinations of temperature and relative humidity effective in killing the coliforms on nut meats in a 30-minute period were as follows: 150°F. and 90 per cent, 160°F. and 80 per cent, and 180°F. and 70 per cent. A combination of 200°F. and 50 per cent was also effective and required less treating time.

Pasteurization at 150°F. and 90 per cent relative humidity altered the texture of the meats, but 160°F. and 80 per cent, also 180°F. and 70 per cent, were not deleterious to either the flavor or the texture. The use of 200°F. and 50 per cent relative humidity produced milder flavor and slightly brittle texture. Treatment at 300°F. for 10 minutes produced brittle meats almost devoid of the characteristic walnut flavor.

It is suggested that a relative humidity of 50 to 70 per cent be used as one of the factors of the pasteurization procedure. With such relative humidities the operation of the kiln is facilitated, the original moisture content of the nut meats is altered but little, and danger of mold growth in storage is small if the nuts are of proper dryness before processing.

Differences in initial moisture content of the nut meats affected the rate of killing the bacteria on them.

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CONTROLLING QUALITY CHANGES IN CURED MEATS BY PACKAGING [1]

W. M. URBAIN AND J. M. RAMSBOTTOM
Research Laboratories, Swift & Company, Chicago, Illinois

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A major problem for producers of certain meat items is maintenance of quality during the time between production and consumption. Meat items which involve special problems are those which undergo extra preparation for the consumer, such as slicing, small-unit packaging, cooking, etc. Sliced bacon, sliced ham (boiled or smoked), sliced dried beef, and frankfurts may be cited as examples. Quality changes which are likely to occur include loss of normal appearance owing to discoloration, to dehydration, or to chemical or microbiological action; and flavor losses or changes owing to a variety of factors.

The food producer, and to some extent the consumer, has certain general avenues of attack he can follow in order to maintain initial quality. They may be stated briefly as follows: (1) the period between production and consumption may be shortened and the handling improved; (2) the composition of the products may be changed in order to secure a more stable product; and (3) packaging measures may be taken to provide maximum protection of the product. Of these general approaches, the first two have inherent and long-standing limitations which largely preclude obtaining any major improvement through them. Generally speaking, distribution requirements and consumer convenience demand a period of some days between production and consumption. While this period logically is to be held to a minimum, some of the products in question are by common usage expected to have an appreciable life; in fact, their preparation originally had that as an objective. Sliced dried beef is an example. The availability of refrigeration and present meat-handling procedures lessen the need for anything but a normal life for meat products; nevertheless some of the old customs continue. These practices run into direct conflict with formula changes, which, in line with the availability of refrigeration, have tended to "milder" cures of salted products, such as dried beef and bacon. Low temperatures rather than high salt contents have become the main preservative. The combined effect of the two tendencies, namely to regard certain meat items as having relatively long life and to alter formulas in a way which reduces product life, is to accentuate the problem of initial quality maintenance. Certainly the fact that these tendencies exist makes it not too likely that improved quality maintenance between production and consumption can be accomplished through these channels.

The third general measure for protecting quality, namely packaging, appears to offer an approach which does not run into conflict with established usage and distribution requirements and at the same time need not involve formula changes.

[1] Presented at the New York meeting of the American Chemical Society, 1947.

Packaging may serve more than one purpose, but its chief function is protection. Up to a recent date this protection function has emphasized sanitary aspects. Present trends add features of product-quality maintenance, largely as a result of the availability of new materials for packaging. To a very large degree it is now possible to select a material which will provide a set of properties in accord with the particular needs.

It is possible to have a logical basis for the selection of a particular packaging material through a knowledge of the chemistry and microbiology of the deteriorative reactions involved in quality loss in a particular product. This paper proposes to demonstrate this thesis by several examples drawn from packaging studies on certain of the meat items previously referred to.

CURED MEATS

These studies were limited to a group of meats which fall in the general class of cured meats. They are marketed unfrozen. We shall proceed by first examining cured meats in general in order to characterize them and determine the general properties of a packaging material to fit their needs.

Cured meats are normally certain cuts of beef and pork which are salted and subjected to special processing. Their appearances and flavors are distinctive. A number, such as bacon and dried beef, are frequently marketed after slicing. Others, such as boiled ham and frankfurts, undergo cooking as part of the regular processing. These operations are apt to reduce the life of the products in question. Generally speaking, these products will have a moisture content below that of fresh meat. Dried beef, which is dried to the greatest extent, will approximate 50 per cent moisture and hence one must recognize that we are still dealing with high-moisture-level products. Salt added in curing will vary from one per cent in bacon to 10 per cent in dried beef. The perishability of these products involves not only microbiological action but also chemical changes and flavor losses. Dehydration, if not prevented, will also affect quality.

The major considerations for maintaining product quality of cured meats may be given as follows:

Normal Appearance: Discolorations owing to dehydration can be controlled by use of a suitable wrapper. Obviously, one completely impermeable to moisture would be most effective. Actually, other factors, such as will be discussed under microbiological action, generally prevent the use of a material which completely seals in the moisture. Regarding this factor each product and each package must be considered individually.

Discolorations owing to chemical action present a serious problem for cured meats. The pigments of cured meats are nitric oxide derivatives of the pigments hemoglobin or myoglobin. Unlike the fresh meats they are quite susceptible to oxidation by atmospheric oxygen. This oxidation is greatly accelerated by light. The oxidation products are brown and of low tinctorial power. The meat, therefore, turns brown or grey. For nitric oxide hemoglobin, the reaction may be written as follows:

$$\underset{Fe^{++}}{NO \cdot Hb} + O_2 \rightarrow \underset{Fe^{+++}}{Met\ Hb}$$

As a result of the sensitivity of the cured-meat pigments to atmospheric oxygen, packaging considerations must include some measure of protective action for the color. Where applicable, exclusion of light through the use of opaque materials is quite effective. Where opaque materials are not acceptable, exclusion of oxygen seems to be the only other procedure. In general, where storage periods are long, this latter measure is indicated regardless of the opacity of the wrapper.

Microbiological action affecting appearance of cured meats may be divided grossly into two types—mold and bacteria. Mold ultimately produces visible growths which are objectionable. Bacteria produce "slimes." As far as packaging is concerned, effective measures will vary depending upon the product. Exclusion of oxygen prevents mold growth. Reduction of humidity or surface moisture controls both bacterial and mold growth, at the risk, however, of dehydration. Frequently a compromise which provides a "semi-moisture-proof" material is indicated.

Flavor Losses and Changes: Generally speaking, it can be said that protection of appearance results in conditions which aid in flavor retention. Prevention of moisture loss, control of microbiological action, prevention of escape of flavor through evaporation, and prevention of oxidation all aid appearance and flavor simultaneously. There are, of course, exceptions to this generalization. For example, simple oxidation of the meat pigment of bacon may not affect flavor, although it will adversely affect appearance. Almost without exception, however, cured meats which have a good normal appearance will also have good flavor.

EXPERIMENTAL STUDIES

The advantages of a particular wrapper are best determined by comparison with other materials. A few specific studies are given here which will illustrate this procedure and at the same time indicate certain of the requirements for specific products.

Studies on Sliced Bacon: At present sliced bacon is generally marketed in one-half or one-pound units. Suitable paper or normal cellophane are common wrapping materials. Conditions may vary greatly, but a product life of from several days to several weeks may be required. During this period storage is usually under refrigeration temperatures of —1.1 to 10°C. (30 to 50°F.). During distribution the product may be subjected to varying temperatures.

Changes in normal appearance include color changes through oxidation and/or dehydration and, under extreme conditions, development of visible mold growth. Flavor changes include loss of the normal smoked flavor, development of fermented or sour flavors, rancid flavors, and moldy flavors. The time schedule for these changes varies with conditions.

Generally speaking, with normal commercial handling appearance will hold up for two to three weeks. Flavor losses may become significant in about two weeks. This generalization applies to most of the common packaging materials in use at present.

A study was made comparing two wrapping materials for sliced bacon. These wrappers differed essentially in one respect—in the one package air was permitted access to the product; in the other, all air was removed and the package sealed, the wrapping material being impermeable to oxygen.

Results obtained with a test panel on flavor changes over a period of time are shown (Fig. 1). In this test the cooked bacon was scored on a basis of 2.0 for perfection, 1.0 for fair quality, and 0.0 for poor quality. Bacon from the same slab was employed in the comparison of the two wrappers. At each eating test, the test panel was "calibrated" by a sample of freshly sliced bacon (from a slab different from that used for the wrapper-test samples). All samples were identified by a code not known to the testers.

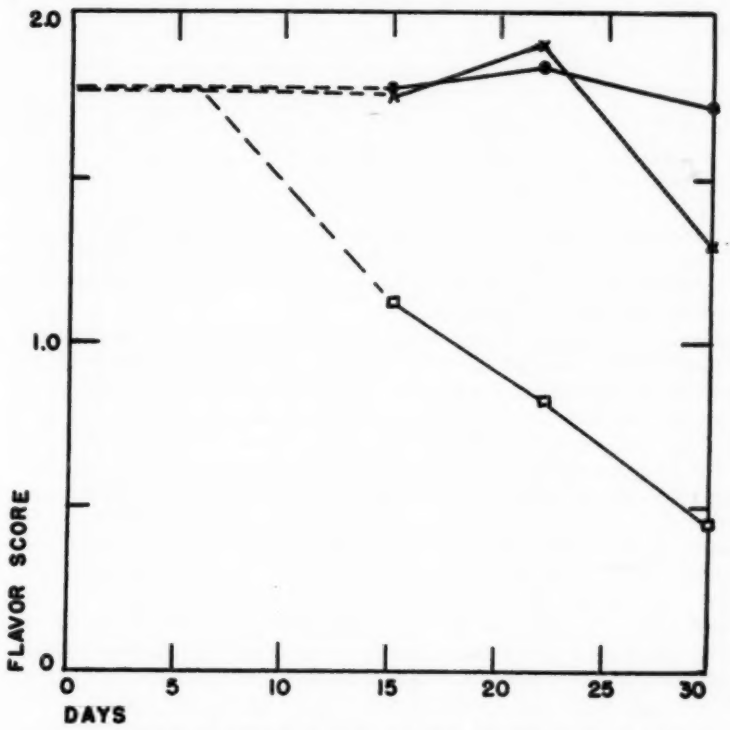

FIG. 1. Effect of exclusion of oxygen on flavor retention of sliced bacon.
●——● Fresh bacon.
X——X Vacuum-packaged bacon.
□——□ Normal cellophane-wrapped bacon.

As can be seen (Fig. 1), exclusion of oxygen resulted in significantly improved flavor retention. The test also showed that simultaneously color retention was perfect and mold growth eliminated.

Studies on Sliced Dried Beef: Sliced dried beef is a product of approximately 50 per cent moisture and 10 per cent salt. As might be anticipated from this composition, it is quite stable. Custom, however, takes advantage of this stability and permits practices which amount to abuse. For instance, this product is not always kept under refrigeration.

Product life is expected to be long—a month or more. Stoppage of flavor loss plus protection of the bright red color constitute the main problems in packaging sliced dried beef.

Results of a study of several wrappers in connection with these problems are given (Table 1). In these tests, after slicing, the product was held under conditions simulating distribution and consumer handling. The first examination was made after three weeks and additional observations were made for a number of weeks. These observations were simple inspections by a group of product experts. It can be seen (Table 1) that

TABLE 1

Sliced Dried-Beef Studies

Wrapper	Normal cellophane	Pliofilm	Polyethylene	Aluminum foil	Vacuumized package
Color retention	Fair	Fair	Fair	Poor	Good
Flavor retention	Fair	Fair	Fair	Fair	Good

the vacuumized package provided the best protection. This is in line with the well-established success of glass jar, vacuum-packed, sliced dried beef which has been a market item for many years and in which color retention has been no problem.

Studies on Fading Prevention: Mention has been made of the effect of light on the color of cured meats. In the above-described studies on sliced bacon and sliced dried beef, the products were exposed to light of intensity and duration no greater than ordinarily encountered in what has been the normal course of marketing such products. Certain new trends in merchandising, however, are apt to expose such products to more rigorous conditions. For example, the refrigerated display case has been developed to permit self-service for meat items. These display cases usually have a high level of illumination directed at the products they contain. While fresh meats are little affected, certain cured meats can be faded extensively; for example, sliced boiled ham wrapped in a transparent wrapper, such as normal cellophane, will show distinct fading within two hours and within four hours is badly faded. Several avenues of attack for controlling the effect of light are suggested:

(1) Light may be excluded by the use of an opaque wrapper.

(2) The quality of the light may be altered to render it less effective in fading.

(3) Through the use of a suitably colored wrapper, effect (2) may be secured.

(4) The geometry of the lighting set-up may be altered to reduce the photochemical efficiency of the light.

The absorption curve for nitric oxide hemoglobin is shown (Fig. 2). Assuming that light which is absorbed is photochemically active, it is clear that all but red (and ca. 600 mμ and beyond) of the spectrum will cause fading. In the presence of oxygen any use of a generally transparent wrapper will, therefore, have to be coupled with the use of a light source emitting only red light. The use of a red light has not been investigated because of its unsuitability from a merchandising viewpoint.

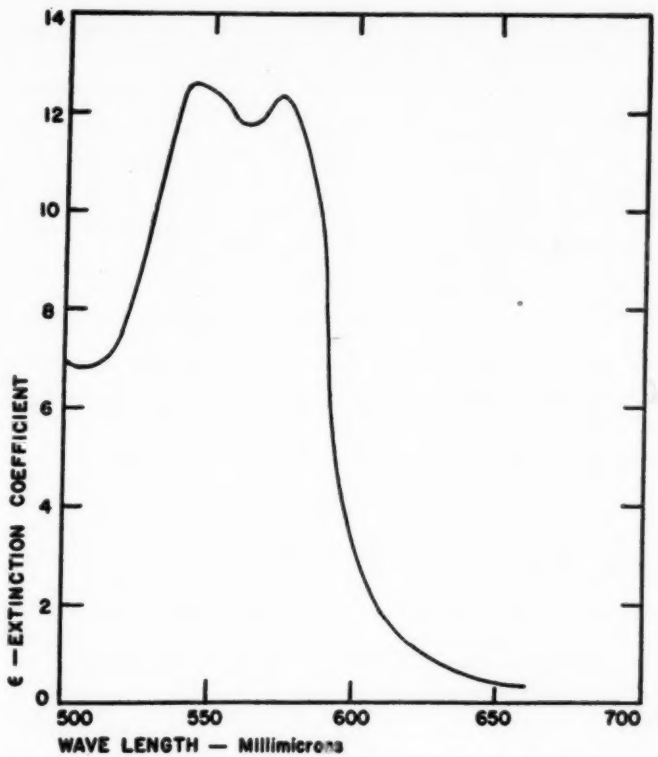

FIG. 2. Absorption spectrum of nitric oxide hemoglobin [data of Urbain and Jensen (1940)].

The use of colored wrappers, which theoretically could be as effective as a colored light, was investigated. A number of portions of sliced boiled ham were wrapped in semi-moisture-proof cellophane and one each overwrapped with (1) semi-moisture proof cellophane, (2) amber normal cellophane, and (3) red normal cellophane. These were exposed to fluorescent lights in a refrigerated display case where the light level on the products ranged from 40 to 50 foot candles. Inspection after six hours revealed that all packages showed discoloration. Inspection after 20 hours showed the red-cellophane-wrapped ham to be substantially better than the others, confirming theory.

The effect of different angles of incidence of light upon the fading reaction was investigated by holding sliced boiled ham, wrapped in normal cellophane, under fluorescent lights ("soft white light" type) at temperatures of about 1.7°C.(35°F.) and observing the degree of fading.

The data show that a substantial improvement is possible when large angles of incidence are employed compared with more usual low angles (Table 2). The control sample of the series of packages referred to (Table 2) was held in the dark and demonstrates the value of an opaque wrapper.

TABLE 2

Effect of Angle of Incidence of Illumination on Fading of Sliced Boiled Ham in Normal Cellophane

Incidence angle	Fading at			
	2 hr.	4 hr.	7½ hr.	14½ hr.
0°	Distinct	Strong	Very strong	Very strong
19	Distinct	Strong	Very strong	Very strong
34	Slight	Strong	Very strong	Very strong
43	Slight	Strong	Very strong	Very strong
92	Very slight	Distinct	Very strong	Very strong
103	Very slight	Distinct	Strong	Very strong
119	Doubtful	Distinct	Strong	Very strong
143	None	Slight	Slight	Very strong
160	None	None	Slight	Very strong
180[1]	None	None	Very slight	Strong
Control[2]	None	None	None	None

[1] Cardboard back of package toward light. [2] Wrapped in opaque material.

Recalling that the fading effect is due to acceleration of the reaction

$$NO \cdot Hb + O_2 \rightarrow Met\ Hb,$$

a simple solution for the problem of fading is to exclude oxygen, thereby eliminating one of the reactants. Vacuum packaging offers this as a possibility.

Results of an investigation in which sliced boiled ham was packaged so as to determine the effect of exclusion of oxygen upon fading of color are shown (Table 3). The product was held under fluorescent lights ("soft white light" type) at 2.2 to 5.6°C.(36 to 42°F.).

TABLE 3

Effect of Exclusion of Oxygen Upon Color Fading of Sliced Boiled Ham

Package	Light	Color score[1] at					
		0 days	1 day	3 days	5 days	8 days	13 days
Semi-moisture-proof cellophane	Yes	10	4	2	2	1	1
Semi-moisture-proof cellophane	No	10	10	10	9	9	7
Transparent wrapper vacuumized	Yes	10	6	8	9	9	9
Transparent wrapper vacuumized	No	10	10	9	9	9	9

[1] Based on 10 equals "perfection," 1 equals "extremely poor."

From a packaging viewpoint the data (Table 3) indicate that color protection of a product such as sliced boiled ham is secured best through exclusion of light or through exclusion of oxygen. These results are those predicted from a knowledge of the chemistry of the fading reaction.

It is interesting to note the figures (Table 3) referring to the vacuumized package. Within 24 hours a color score of six was recorded, indicating extensive fading. At the end of three days this had risen to eight and within five days had returned almost to the initial value of 10. This

rather mystifying series of changes is an indication of the extreme sensitivity of meat pigment to oxygen. An explanation of the changes may be given as follows:

The vacuumized packages contain a small residuum of oxygen. This, under the catalytic influence of the light, causes oxidation of the pigment. Since the package is sealed, no additional oxygen can get in. The pigment oxidation reaction, plus the normal "biological oxygen demand" of the ham, consume the initially entrapped oxygen, eventually producing anaerobic conditions. Through continued effect of the normal biological oxygen demand the pigment is reduced, and combines with the curing agents (always used in slight excess) to form the initial pigment, thereby restoring the original normal color of the ham.

OTHER FACTORS INVOLVED IN SELECTION OF A PACKAGE

It is recognized, of course, that the final selection of a wrapping material depends not only upon the factors discussed in this paper but also upon many others. Some of these other principal factors may be listed as follows: (1) durability; (2) safety, e.g., from a bacteriological viewpoint; (3) cost; (4) suitability from an operations viewpoint; (5) suitability from a merchandising viewpoint.

The choice of a wrapper for a particular product will be based on technical requirements, such as discussed in this paper, balanced with other factors, such as listed above.

REFERENCES

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DEPENDABILITY OF FOOD JUDGES AS INDICATED BY AN ANALYSIS OF SCORES OF A FOOD-TASTING PANEL[1]

ANDREA OVERMAN
Department of Home Economics, Oregon Agricultural Experiment Station
AND
JEROME C. R. LI
Department of Mathematics, Oregon State College, Corvallis, Oregon

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Every laboratory engaged in food research or development encounters the problem of how best to evaluate food products. Objective tests are few, and limited in their application. Dependence must be placed on subjective methods for estimating the quality of foods.

The method most frequently used in food research laboratories is the bringing together of a number of persons to form a tasting panel, as mentioned by Crist and Seaton (1941), Monerieff (1947), and others. The panels have as their purpose a screening action, a sifting and sorting of the better food products from those that are fair or poor. The panels may act, therefore, to guide the lines of inquiry in the laboratory, and their judgments may be influential in bringing a product to or withholding it from the market. Though there are obvious advantages to be gained from securing the opinion of many persons, tasting panels are usually very small. Platt (1931) says, "Five or even ten judges are good for important conclusions if that number of qualified persons are available."

Certain assumptions must be made in regard to this type of panel: (1) Since the number of persons on the food-scoring panel is small, those who are panel members must have exceptionally good tasting ability. (2) Though the number of persons is small, their combined judgments represent general consumer preference.

The second point cannot be determined without a direct comparison of the preferences of the scoring committee with those of the general public. This may be done after preliminary screenings have been made. The first point is subject to ready analysis, since, if differences exist among the foods, a good judge should be able to discriminate among foods which are known to have different qualities, and he should be able to reproduce his judgments. The judge who does not detect differences, as well as the one who cannot duplicate his own judgments, should be removed from the panel.

Techniques for organizing small tasting panels have not been standardized, either in regard to the selection of the panel or its training. The members of the panel are frequently selected for their interest or their availability, rather than for the acuity of their senses of taste and smell.

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It is seldom feasible to train such a panel to detect specific off-odors or flavors which may exist in the various foods being investigated. This is partly because of the amount of time such training would require of all persons involved, and partly because specific off-odors or flavors can scarcely be anticipated, or if anticipated, no standards for comparison may exist.

Until the time arrives when standards are established for all kinds of foods, and the techniques involved in panel testing are uniform, some methods will be needed for comparing the members of the tasting panel as to the reliability of their judgments.

The evaluation of food-tasting scores to determine whether a person is a good judge involves finding (1) whether the judge can discriminate among foods of the same kind which are known to differ in flavor or other qualities, and (2) whether he can duplicate his own judgments. For such determinations, judgments on replicate lots of foods are necessary.

Information for evaluating the abilities of judges on a small food-tasting panel is presented herewith. Rather than listing recommendations and suggestions as such, steps in the actual analysis of scores given by a panel of judges are narrated in detail. For conditions stated or implied, the procedures presented may be taken as recommendations.

The panel used as an example for analysis was composed of ten persons. Each judged the quality of pastry made with five fats which were known to have different flavors.

EXPERIMENTAL PROCEDURE

Selection of the Panel: Following a common procedure, the members of the tasting panel were selected chiefly because of their availability. No effort was made to determine taste or smell thresholds, nor were the judges trained to evaluate flavor defects of fats.

Method of Scoring: Since all these persons could not conveniently be present at the same time, a procedure was followed that would enable them to come in whenever they wished during each day of the testing. The method of silent, individual scoring was used. Small tables were placed at some distance from each other, and arranged to seat one person. Samples of pastry were arranged on wire cake racks, with the numbers of each sample written clearly on a sheet of paper placed beneath the rack. Score sheets, napkins, and water were placed beside the rack.

Five samples of pastry, each made with a different fat, were presented to the panel for judging on each of ten consecutive days (one weekend out). In this way, ten judges scored ten replicate lots of pastry made with each of the five fats. The order of the pastry samples was changed each day. All possible variables in the preparation of the pastry were controlled so that differences were due only to the different fats used.

Every judge scored the pastry every time.

Score Sheets: The score sheets were simple and brief. Descriptive terms were used for the different characteristics judged. That is, the pastry was judged for its crispness, tenderness, and flavor, and each of these characteristics was rated excellent, good, fair, poor, or not edible by the judge. After the judging, numerical values were assigned each

rating. The ratings and their values were excellent, five; good, four; fair, three; poor, two; not edible, one.

Separate sheets were provided for scoring each sample. Another sheet was provided for rating the samples according to the preference of the judge.

ANALYSIS OF SCORES

The methods used for analyzing the scores were selected to yield information about each judge's ability to discriminate between the different samples and to reproduce his own judgments.

A preliminary study of the data was made by means of simple methods to ascertain some of the more obvious differences among the judges. The analysis of variance was used to obtain more accurate information about the reliability of each judge.

PRELIMINARY STUDY OF DATA

Measures obtained by the preliminary methods described in this section can be only rough indications of judging ability. The methods are useful, however, particularly if the services of a statistician are not available, for they permit a rapid classification which serves to identify the best and the poorest judges.

Consistency: Two methods which provide rapid measurements of consistency are suggested here. One is a comparison of the average range of each judge's scores; the other a comparison of the number of times each judge duplicated his scores for each kind of pastry.

The ease with which the range, or the number of duplicate scores, can be ascertained from a simple tabulation of scores as shown (Table 1), may be illustrated with the scores given by Judge A to pastry made with Fat Number I.

Fat	Judge	Scores for replicate lots of pastry										Range	Duplicates
		Lot No.	1	2	3	4	5	6	7	8	9	10		
														4 - 3's
														2 - 4's
I	A	Score	5	3	5	5	4	3	3	5	3	4	2	4 - 5's

The lowest score was subtracted from the highest score to find the range. Each score was counted to find the number of duplications.

The ranges of scores given by Judge A to pastry made with each of the five fats were 2, 2, 2, 3, 2. The average range was 2.2.

The average range for each of the judges was as follows:

Judge	A	B	C	D	E	F	G	H	J	K
Average range	2.2	2.4	1.4	3.0	1.6	2.6	2.4	1.2	1.8	2.0

The judges having the smaller average ranges would be considered the more consistent. Judge H, with an average range of 1.2, was most consistent; whereas Judge D, with an average range of 3.0, was least consistent.

TABLE 1

Judge's Scores for Pastry Made With Five Fats Having Different Flavors[1]

Fat	Judge	Replicate lots										Total
		1	2	3	4	5	6	7	8	9	10	
I	A	5	3	5	5	4	3	3	5	3	4	40
	B	3	5	5	5	4	5	4	5	4	4	44
	C	3	4	3	4	4	4	4	4	4	4	37
	D	4	2	1	4	4	4	2	4	2	4	31
	E	5	4	4	4	4	4	4	5	4	5	43
	F	4	4	4	3	3	4	4	4	3	5	38
	G	5	4	5	5	4	5	4	3	5	5	45
	H	4	4	4	4	4	5	5	5	5	5	45
	J	4	3	4	3	2	4	3	4	3	4	34
	K	5	5	4	3	4	4	4	5	4	5	43
II	A	4	5	4	5	4	5	4	4	4	3	42
	B	4	4	4	4	5	3	3	5	4	3	39
	C	4	3	4	3	4	4	4	4	3	4	37
	D	2	5	2	4	1	1	3	4	3	3	28
	E	5	5	4	4	5	4	4	3	5	4	43
	F	3	5	4	4	5	3	3	4	4	4	39
	G	4	3	5	4	5	5	5	5	5	5	46
	H	3	4	3	4	3	4	4	5	4	4	38
	J	3	4	3	4	3	3	4	3	4	3	34
	K	4	5	5	5	5	5	5	4	5	5	48
III	A	4	5	3	3	5	4	5	5	5	5	44
	B	5	2	3	5	3	5	4	2	5	5	39
	C	4	4	4	4	4	4	3	4	4	4	39
	D	5	4	4	4	3	4	4	3	5	3	39
	E	4	5	5	4	5	5	5	4	5	4	46
	F	5	4	3	5	4	3	4	4	5	4	41
	G	4	5	5	4	4	3	5	4	4	5	43
	H	3	3	3	3	3	3	3	3	3	4	31
	J	4	4	4	3	4	2	4	3	4	3	35
	K	5	5	4	4	5	4	5	4	3	3	42
IV	A	3	4	3	4	3	2	5	4	4	3	35
	B	4	4	4	3	5	2	4	4	4	4	38
	C	4	4	4	4	3	2	4	3	4	4	36
	D	1	3	3	2	1	4	1	1	1	1	18
	E	4	4	3	3	4	3	4	4	4	3	36
	F	4	2	4	3	3	1	2	3	4	2	28
	G	5	5	4	3	4	2	4	5	2	3	37
	H	2	2	2	2	2	2	2	2	2	3	21
	J	1	3	3	1	2	3	3	2	3	3	24
	K	4	4	5	5	3	2	3	4	4	4	38
V	A	2	2	2	2	2	3	1	1	1	2	18
	B	3	2	3	2	2	4	3	3	2	3	27
	C	2	3	4	3	3	4	3	2	2	2	28
	D	4	3	3	3	4	1	1	1	4	1	25
	E	2	3	2	3	3	5	2	3	3	3	29
	F	1	1	1	1	1	5	1	1	2	1	15
	G	2	5	3	3	2	5	2	2	4	2	30
	H	2	2	2	2	2	2	1	2	2	1	18
	J	2	3	1	2	2	3	1	2	1	1	18
	K	2	2	1	2	1	3	1	1	1	1	15

[1] Ten replicate lots of pastry scored for each different fat.

The comparison of average ranges is preferred to other methods for a preliminary evaluation of consistency, since it is most rapid and easy to comprehend.

A tabulation of duplicated scores is interesting, for it yields some information about the tendency of some of the judges to score according to patterns of their own. Some judges tend to score high, some low. Others, who probably cannot distinguish flavor differences, may give scores ranging all the way from "not edible" to "excellent" for the same food, as did Judge D for pastry made with Fat II. Others, perhaps equally in doubt as to flavor differences, will favor scores in the center of the range. For example, Judge C strongly favored the score of four.

Discrimination: Testing a judge's ability to duplicate his judgments is comparatively easy. Finding valid tests for determining his ability to discriminate is more difficult.

Some information about discriminating ability was obtained by means of a tabulation of each judge's average score for pastry made with each fat, together with the sum of the absolute values of his deviations from his own means. This method may be illustrated by the tabulation for four of the judges (Table 2).

TABLE 2

Mean Scores of Each Judge and Sum of Absolute Values of Deviations From His Own Mean Scores for Flavor of Pastry Made With Five Different Fats[1]

Judge and score identification	Fat I	Fat II	Fat III	Fat IV	Fat V	All fats, total deviation
Judge C						
Mean score	3.7	3.7	3.9	3.6	2.8	.....
Sum of deviations from mean	4.2	4.2	1.8	5.6	6.4	22.2
Judge D						
Mean score	3.1	2.8	3.9	1.8	1.9	.....
Sum of deviations from mean	10.8	10.4	5.4	9.6	7.2	43.4
Judge E						
Mean score	4.3	4.3	4.6	3.6	2.9	.....
Sum of deviations from mean	4.2	5.6	4.8	4.8	5.4	24.8
Judge H						
Mean score	4.5	3.8	3.1	2.1	1.8	.....
Sum of deviations from mean	5.0	4.8	1.8	1.8	3.2	16.6

[1] Ten replicate lots of pastry were scored for each fat.

The totals of the deviations, as shown in the column to the extreme right, show a very low deviation of 16.6 for Judge H, and a very high deviation of 43.4 for Judge D. This is in agreement with the finding, through use of the range, of the consistency of these two judges. It is easy to see that Judges C, E, and H were much more consistent in their judgments than was Judge D.

A high deviation from his own mean indicates an inability to duplicate judgments or marked changeability in opinion during the series of tests. Either would be undesirable in a judge.

A low deviation from his own means indicates either a high degree of reproducibility of judgments or a lack of discrimination among different samples. If all scores are high, a low deviation also could mean that

a judge is easily pleased; or if all scores are low, that the judge is highly critical. A third possibility is apparent. If a judge considered two different flavors equally desirable, with the result that he gave both samples an identical score, it is evident that his ability to discriminate would be partially obscured. The fact that he could discriminate would not appear through an analysis of his scores. It should be emphasized that the procedures for obtaining scores, not the analytical methods used, were at fault in not avoiding this error.

The first quality mentioned in the above paragraph—a high degree of reproducibility—would be highly desirable in a judge; whereas, the second quality—an inability to differentiate among samples—would be undesirable in a judge. Whether a judge belongs to one of these classifications may be determined by checking his mean scores for pastry made with each fat. If all or most mean scores are practically identical, a lack of ability to differentiate is indicated. For example, the means of scores given by Judge C to pastry made with the five different fats were 3.7, 3.7, 3.9, 3.6, and 2.8. Apparently, Judge C did not discriminate among products made with four of the fats, but did show through his scores that he considered the fifth to be different and less desirable than the other four.

As has been mentioned, the fact that a judge gives the same high score to four products out of five, may mean that the judge cannot discriminate. It may also mean that the judge is very easy to please. This characteristic, though often desirable in daily living, is not a good characteristic for a judge on a food-scoring panel.

If mean scores are different, and this is accompanied with low variability, as shown by low deviations from his own means, the judge may be considered highly reliable in his ability to detect differences.

ANALYSIS OF VARIANCE

The consistency of the judges and their ability to discriminate may be determined with greater accuracy by a more elaborate statistical method.

Discrimination: The panel of judges was tested by means of the analysis of variance,[2] Snedecor (1946), to determine whether the judges as a whole had discriminated among pastries made with the different fats. The analysis of variance of flavor scores for the group is shown (Table 3).

TABLE 3

Analysis of Variance of Combined Flavor Scores for Pastry Made With Five Different Fats

Source of variation	Degrees of freedom	Variance	F
Fat	4	60.9530	81.93
Judges	9	9.1736	12.33
Error	486	0.7440	.....
Total	499	.....	.....

[2] In this analysis, several assumptions underlying the analysis of variance may not be satisfied, such as the normal distribution of the scores and the homogeneity of the error variances of the judges. This introduces very little error into the significance level of the F-test, Cochran (1947).

The analysis showed that, according to the combined judgment of the panel, pastries made with different fats had different flavors. This is shown by the very high F-value, Snedecor (1946), for variations due to the kind of fat used.

Since discriminating ability was shown to exist in the panel, the logical step to follow was the breakdown of the panel for the purpose of comparing the abilities of individual judges.

The discriminating ability, as well as the consistency of each of the ten judges could be, and was, measured by means of the analysis of variance. Ten replicate lots of pastry were scored for each of the five fats. The numbers of the degrees of freedom for "among fats" and "within fats" (error) are four and 45, respectively. The variance-ratios (F-values) of the 10 judges are given (Table 4).

TABLE 4

The Error Variance and Variance-Ratio of Each Judge's Scores, for Pastry Made With Five Fats Having Different Flavors

	Judge									
	A	B	C	D	E	F	G	H	J	K
Error variance.....	0.6244	0.7533	0.3356	1.3667	0.3978	0.8333	0.8689	0.2111	0.5178	0.5200
Variance-ratio.....	17.65	5.22	5.45	4.37	11.89	14.12	5.14	60.78	11.20	32.06

The ten variance-ratios (F-values) (Table 4) were used as an index to measure the discrimination of each judge. Since F-values for every judge exceeded 3.77, which is approximately the one per cent level of the F-distribution with four and 45 degrees of freedom, it is apparent that all the judges had discriminating ability. That some were more discriminating than others is shown by the difference in magnitude of the F-values. The higher F-values, such as those for Judges H, K, and A, indicate greater discriminating ability, whereas the lower F-values, such as those for Judges B, C, D, and G, indicate less ability to distinguish differences.

Consistency: The ten error variances (Table 4) were due to variations in duplicating judgments during scoring by each judge. They were, therefore, appropriate to use as a measure of the consistency of the judges.

If the error variances differ significantly from each other, the general statement may be made that the smaller variances indicate greater consistency and the higher variances less consistency in the scoring. For example, Judge H, with an error variance of 0.2111 was much more consistent in the scores he gave than Judge D, who had an error variance of 1.3667.

Differences among members of this panel were sufficiently marked so that a test of the panel for homogeneity of variances was not necessary. However, if members of a panel did not show such marked differences in individual error variances, it would be advisable to test the panel for homogeneity of variances before proceeding to compare individuals. If the panel were homogeneous in consistency, then no further testing of individuals would be necessary.

Judges who have been trained, or selected for their judging ability, should approach homogeneity more closely than could be expected of an untrained and unselected group.

Since it is sometimes advisable to test a panel for homogeneity of error variances, the method used is mentioned here, together with an example from the panel used in this study.

The homogeneity of the ten error variances was tested. The method used was Bartlett's test (1937), a description and a numerical example of which is given in Snedecor (1946). Details of the calculation are omitted here.

The chi-squared value was found to be 51.72 with nine degrees of freedom. Referring to the chi-squared table, it is evident that the variances of this panel were heterogeneous; that is, the judges were not homogeneous as far as consistency is concerned.

The consistency of each judge was then compared with the consistency of every other judge by finding the ratio of the two error variances concerned, then testing for significance against the F-table. The five per cent and one per cent levels given in the F-table, Snedecor (1946), are 1.66 and 2.06, respectively. If the larger variance is always divided by the smaller variance to obtain the variance-ratio, the tabulated one per cent and five per cent levels of the F-distribution are actually the two per cent and 10 per cent levels, respectively.

If the ratio of any two judges was less than the chosen significance level, the two judges concerned were equally consistent. For example, the variance of Judge D, divided by the variance of Judge H, is 1.3667/0.2111 = 6.47. This value is far greater than 2.06, which is the two per cent level. This indicates that H was a more consistent judge than D.

DISCUSSION

The use of ranks rather than scores would have encouraged the judges to make finer distinctions among samples, thus eliminating many of the ties found in the scores. The use of ranks would have had the added advantage of reducing the tendency of individual judges to prefer certain scoring ranges; for example, some judges tended to score all samples from one through four, whereas others tended to score from three through five.

If ranks had been obtained instead of scores, the methods of analysis would have been exactly the same, except that the ranks would first have been transformed into normal scores, as given in Table XX, Fisher and Yates (1938).

SUMMARY

Suggestions have been made for comparing the members of a food-tasting panel as to the reliability of their judgments of flavors.

The panel used as an example for analysis was composed of ten persons. Each judged the flavor of pastry made with five fats which were known to have different flavors. Ten replicate lots of pastry were scored for each fat.

Two methods of analysis have been suggested: The first, a preliminary study and evaluation of the tabulated data in which the range, the number of duplicated judgments, and the absolute deviations from means were

used to ascertain some of the more obvious differences among judges. The second method suggested was the use of the analysis of variance to measure the consistency and the discriminating ability of each judge.

A high ability to detect differences, together with a low variability in duplicating judgments, are indications of good judging. Conversely, a high variability, or lack of ability to detect differences, are indications of poor judging.

ACKNOWLEDGMENT

This opportunity is taken to express appreciation for the co-operation of the following persons, who were members of the food-tasting panel: Helen Charley, Joyce H. Dunn, Margaret L. Fincke, Betty E. Hawthorne, Mary Frances Helvogt, Miriam Macpherson, Mildred Riedesel, Demetrios Hadjimarkos, A. W. Oliver, and G. H. Wilster.

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EDITORIAL REVIEW

OBSERVATIONAL STUDY OF MONOSODIUM GLUTAMATE

E. C. CROCKER AND L. B. SJÖSTRÖM
Arthur D. Little, Inc., Cambridge, Massachusetts

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The present considerable interest in monosodium glutamate by food manufacturers makes analysis of its taste timely and useful. This study summarizes some of the early literature on the subject and adds new observations made on the pure monosodium glutamate now commercially available. Comparisons also are made between glutamate, hydrolysate, and meaty flavors, concerning the relationship of which there is much confusion.

The term "glutamic taste" has existed since 1912, when Ikeda [1] used it in describing the taste sensations common to the glutamates of sodium, potassium, magnesium, calcium, and barium, and in part to the free acid. It has always been considered rather difficult to define. The writer, with L. F. Henderson,[2] in 1932 published a short study on the glutamic taste, describing an attempt to duplicate it synthetically, with a mixture of sweetness, saltiness, sourness, and bitterness. It was found possible at that time to match the taste of monosodium glutamate fairly well at two times threshold intensity (0.0050 M of glutamate, or 0.084 per cent) using 0.6 of a threshold of sweetness, 0.3 of sourness, 0.7 of saltiness, and 0.9 of bitterness. When any one of these taste components was varied appreciably, the match was then noticeably less exact. The "threshold" used for sweetness was 0.020 M (0.684 per cent) of pure sucrose; that for saltiness, 0.030 M (0.176 per cent) of sodium chloride; for sourness, 0.00125 M (0.019 per cent) of tartaric acid; and for bitterness, 0.00020 M (0.00424 per cent) of caffein.

Glutamic acid is structurally almost unique among the amino acids. Only aspartic, hydroxyglutamic, and glutamic acids have a second carboxyl group in addition to the one adjacent to the alpha amino group. This feature makes for a degree of acidity not found among the other amino acids and gives these acids the capacity for making sodium salts with approximately neutral reaction. Glutamic acid may exist in several different ionic forms, depending upon the concentration and the presence of other ions, but principally upon the acidity or alkalinity of the solution. Examination of a hydrochloric acid titration curve indicates that glutamic acid forms a hydrochloride at pH about 0.7, the free acid at pH about 3.2, and the monosodium salt at pH about 7.0.

On adding sodium hydroxide, it is found that the disodium salt exists at about pH 12.0. Several modern samples of high-grade monosodium glutamate when dissolved in neutral water at 0.3 per cent concentration,

[1] Ikeda, K., 1912. Orig. Com., Eighth Int. Cong. Appl. Chem., **18**, 147.
[2] The American Perfumer **27**, 156 (1932).

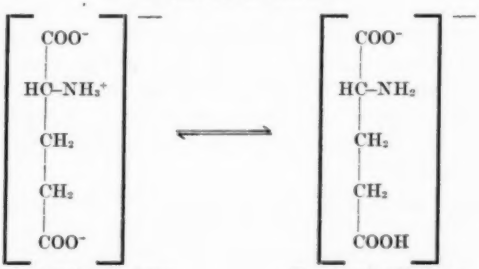
gave pH values averaging 7.0 ± 0.1, indicating that they are predominantly the monosodium salt. Since the pH of most cooked foods is generally lower than 7.0, it is possible that several types of glutamate ion may be present and responsible for the taste and flavor effects noted in the food. While some foods have high values of pH, such as hominy at pH 7.1, and some sauces and relishes low values, in the vicinity of pH 3.5 to 4.5, most of the cooked dishes we have measured fall in the range of pH 6.0 ± 0.5. It would be interesting from a physiological standpoint to determine which ion or molecule is responsible for the glutamate taste and effect.

If the formula for glutamic acid is expressed as H_2G, the conventional ionization equation for monosodium glutamate may be expressed as follows:

$$NaHG \rightleftharpoons Na^+ + HG^- \text{ and } HG^- \rightleftharpoons H^+ + G^=$$

From consideration of the titration curve and the ionization constants of various ions, it may be deduced that the monoacid glutamate ion is the most probable candidate for the production of effects on flavor. Because glutamic acid is an amino acid, the monoacid glutamate ion (HG) may exist in several forms, two of which are shown below[3]:

MONOACID GLUTAMATE ION



A. "Ammonium" form. B. "Amino" form.

At pH 7, the most probable form for the monoacid glutamate ion is Formula A above. This is the so-called "ammonium" form of ion in which the α-amino group is neutralized by the adjacent carboxyl group, which is stronger of the two carboxyl groups, and results in a monovalent ion. In foods at pH of 6.0 ± 0.5 the ion represented by Formula A is probably the predominant ion, and the one responsible for the glutamic taste and glutamate effects on flavor. It is thus argued that the taste and flavor effects of L-glutamic acid are due to the monoacid glutamate ion HG^- ($C_5H_8NO_4$) in one of the several forms in which this ion may exist, such as the "ammonium" form expressed in Formula A above.

Formerly, all amino acids that rotated light to the right were called dextro- or d-acids, and those to the left, levo- or l-acids. Structural investigation has proved that all of the amino acids derived from proteins have

[3] Cohn, E. J., and Edsall, J. T., 1943. Proteins, Amino Acids, and Peptides, Rheinhold Publishing Corporation, New York, New York. P. 94.

the levo-structure, although some, including the important glutamic acid, and lysine, rotate light to the right. At one time, amino acids were provided with a double prefix, having a letter to show the type of structure and a sign to show the direction of rotation. But because there was no generally accepted convention regarding the solvent to be employed for optical rotation observations, much confusion developed. Recently new rules of nomenclature advocate the use of a single symbol prefix denoting configurational family only.[4] Thus, natural glutamic acid, first designated d^-, then $l\ (+)^-$ is now L-glutamic acid. Glutamic acid of D-structure may be produced by resolution of the DL- or racemic acid of synthetic production, through crystallization of certain of its alkaloidal salts.

In 1936, two Japanese investigators, Yanagawa and Nishida,[5] reported tastes of the optical isomers of each of ten amino acids, some 19 chemical individuals in all (glycine is not optically active). These substances were reported as sweet, very sweet, slightly bitter, or no taste, except for dextrorotatory glutamic acid, which was called "nice tasting." Levorotatory glutamic acid was said to have no taste. Dextrorotatory glutamic acid (and its salts) thus stood out as unique in taste among the ten amino acids investigated: glycine, alanine, valine, leucine, phenylalanine, serine, aspartic acid, glutamic acid, histidine, and tryptophane.

Recent tasting by us of single amino acids has shown the taste to be either negligible or a mixture of sweet and bitter. Three acids, glycine, alanine, and hydroxyproline, were found to be conspicuously sweet. The free acids, glutamic and aspartic, and the monohydrochloride of L-lysine, tasted sour because of the presence of many hydrogen ions. The salts of both isomers of aspartic acid were found to be practically tasteless, as was the sodium salt of D-glutamic acid. Only L-glutamic acid and its salts had the "glutamic taste."

Methionine, a sulfur-containing amino acid, varied in odor from very slight to garlic-like, according to the origin and presumably the degree of purity. When the DL-material was purified by treatment of a two per cent solution with decolorizing carbon, practically all odor was removed, and the taste became sweet plus bitter. If held for some minutes in the mouth, this solution gradually became cheesy-tasting, perhaps due to enzymes of the saliva acting upon it. The odoriferous material removed by the carbon was of predominantly acidic nature. Apparently, the pure L- and D-methionines can have no more than sweet and bitter tastes, for the racemic mixture showed these tastes only, and no odor.

A mixture was made for tasting of those amino acids considered essential for human nutrition, plus cystine, in the proportions described by Harte and Travers.[6] All natural isomers were used, of good but not exceptional purity.

These acids were individually finely pulverized in a mortar, weighed, and then intimately mixed together.

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[4] Chemical and Engineering News 25 (19), 1364 (1947).

[5] Reports of the Imperial Research Institute, Osaka (Japan), 16 (10), 1-26 (1936).

[6] Harte, R. A, and Travers, J. J., 1947. Science 105, 15.

Six people tasted the dry mixture in small to moderate quantities, when it was freshly made and again when ten weeks old. All found it sweet, garlic-like, and more or less bitter. Several described it variously as astringent, numbing, sharp, maple-like, caramel-like, or burnt, and with a cheesy after-taste. The flavor, while easily perceived, was mild compared with that of several protein hydrolysates tasted. The flavor intensity appeared to increase on aging, though not markedly. Sweetness was a prominent factor on tasting small portions, but bitterness was quite notable when a large portion was placed on the tongue.

Only one ester of an amino acid has been investigated for flavor—the hydrochloride of the ethyl ester of glycine. It had a weak, sweet odor suggestive of a phenylacetate, and an intensely sour, bitter, and sweetish taste.

TABLE 1

Amino Acid Composition Used for Tasting

Cystine	0.2
Isoleucine	1.2
Leucine	1.7
Lysine	0.8
Methionine	0.5
Phenylalanine	1.4
Threonine	1.0
Tryptophane	0.4
Valine	1.1
Total	8.3

The odor and taste were studied of samples of monosodium glutamate of various degrees of purity. Old, crude materials, such as an authentic sample of Japanese "Aji-no-Moto," tasted strongly salty and had a distinct caramel and gassy aroma similar to that of most protein hydrolysates. "Aji-no-Moto," meaning in Japanese "the essence of taste," was the original commercial form of monosodium glutamate, produced by the process of Ikeda. This particular sample was purchased in this country in 1926. It contained considerable salt in addition to dark-colored and highly flavored impurities. Recent commercial glutamates are only mildly salty and almost completely free from hydrolysate odor and taste. Since pure monosodium glutamate has no odor, but produces only taste effects in the mouth, these effects may properly be referred to as the "glutamic taste," or more generally, as the "glutamate taste."

When an impure monosodium glutamate was agitated in water solution with two per cent of its weight of a decolorizing carbon, a considerable part of the odor (and color) was removed by the carbon. The carbon, after washing with water, was noted to have an appreciable odor of the type distinctive of most crude glutamates, indicating that the odor was adsorbed reversibly. When such an odor-impregnated carbon was treated with dilute sulfuric acid, weak caramel-like acidic substances were set free, suggestive among other things, of the odor of caproic acid. When treated with dilute caustic soda, amines were given off, of piperidine, earthy-animal, and indolic types. Thus, the gelatine-like, or bouillon-like

odor of a crude glutamate is indicated as due to traces of both acidic and basic substances, not completely separated from the glutamic acid or the sodium salt. Numerous tests of pure versus impure glutamate in food preparations indicated that the pure substance usually brought out all the desirable flavor of the food that the impure did, and itself was less intrusive in doing so.

In summation, the very purest monosodium glutamate has been found to be free of odor, but to possess true taste and feeling sensations. The true taste is primarily sweet and salty and secondarily sour and bitter. It might be called "sweet saltiness." This taste is very persistent; the feeling sensation is also persistent. When tasted in quantity, there is a slight tingling produced, especially near the tip of the tongue, as though a weak local anesthetic had been applied. The feeling in the mouth is less easily described: it may also consist of tingling, accompanied by sweet and salty taste, a slight irritation high in the back of the throat, and a desire to loosen any phlegm present. The flow of saliva is stimulated. The mouth feels as though it had been occupied with much eating, leading to satisfaction, but if used in excess, also to dryness. The less pure glutamates, originally sold, had caramel-like "metallic," "gluey," "ossein," or putrid odors, due, no doubt, to the taste of fragments of cracked amino acids superposed on the taste of the pure substance.

RELATIONSHIP OF HYDROLYSATE AND MEATY FLAVORS TO GLUTAMATE TASTE

For centuries protein hydrolysates have been used as a source of flavor in the Orient. Soy sauces of the type called "Sho-yu" by the Chinese were formerly made by the proteolytic digestion of soy beans, using mold cultures. In more recent times faster-acting and more uniform hydrolysis by the use of acid has largely replaced the mold method and wheat gluten, as well as soy beans, is used as the source of the protein. Large quantities of protein hydrolysates are used in the preparation of soy sauces and some is also exported to serve as the base of Worcestershire sauce. These sauces[7] contain the breakdown products of proteins, including peptides, peptones, carbohydrates, and some amino acids, all dispersed in an 18 per cent solution of salt. Protein hydrolysates are now made in this country, in dry form and essentially free from salt, from grain proteins, casein, or lactalbumin. These are principally used as auxiliary nutrients in special diets and as flavoring components of certain food preparations.

Hydrolysate flavor comprises both odor and taste. The odor is usually high in fragrance and burntness and can be compared in this respect with caramel and even licorice. It is also like the highly-heated brown part of roasted meat or the juice, suggesting the use of hydrolysate for the flavoring of gravies. The taste has saltiness and usually considerable sweetness, as well as bitterness and some sourness. If the concentration is high, there is a feeling of astringency. Hydrolysate flavor is like cooked-food flavor in general, with odors and tastes that simulate not only those of meats but probably of all other protein-containing foods, including fried potatoes

[7] "Soya Sauce Processes" by Minor, Lewis J., 1945. Food Industries 17, 758.

and fried and roasted nuts. The odor part of hydrolysate flavor is due to the presence of substances other than amino acids.

Our studies of the flavor of meats cooked at low temperatures [8] and of meat juices indicate that the characteristic feature of meat flavor is the odor or aroma due to the action of heat on the fiber. The taste of cooked meat, noted when the nostrils are closed, is mostly salty and sweet. While taste contributes much to the palatability of meat, one must use his olfactory sense to appreciate meat flavor fully.

Glutamate, as has been pointed out, has taste but no odor. In this it differs from hydrolysates and meat, the flavor of which is made up of both taste and odor. Our work on meat cooked at low temperature has shown that characteristic and identifying factors in meat flavor are odors and that taste components are mainly sweet and salty. Sweet and salty taste is common to most cooked foods and is not an identifying characteristic in any of them. Glutamate has sweet and salty taste in common with meat and most other cooked foods, but this does not imply that it is capable of producing meat flavor, for which it lacks the most important part, namely, the odor. From our study of monosodium glutamate in a wide variety of foods, we must conclude that the concept that it is a meat flavor is erroneous and came about from its association with hydrolysate type of impurities capable of contributing meat flavor components. Howe and Barbella,[9] in an experimental study of meat flavor, also investigated the role of glutamate; they have likewise concluded that glutamate does not possess meat flavor: "Several commercial products (monosodium glutamate) used as meat flavoring substitutes have been purified and recrystallized many times. This recrystallized material has been offered to judges in solutions of high molarity and also in dry form, and meat flavor has not been associated with them. It is our belief that this alleged meat flavor, if present in the original compound, must be due to the impurities present rather than to glutamic acid or its monosodium salt."

We have found the taste of monosodium glutamate to be unique. It is pleasant and is compatible with the flavor of many foods, which it enhances or intensifies when used in concentrations at which its own taste is not obvious. The use of glutamate in food is a large subject, worthy of detailed consideration.

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[8] "The Flavor of Meat," Crocker, E. C.

[9] "The Flavor of Meat Products" by Howe, Paul E., and Barbella, Nicholas G., 1937. Food Research 2, 201.

VITAMIN C CONTENT AND JUICE QUALITY OF EXPOSED AND SHADED CITRUS FRUITS

J. R. WINSTON AND ERSTON V. MILLER

Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

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That the vitamin C (ascorbic acid) content of a fruit is dependent upon the intensity of incident light was suggested by findings of Zilva *et al.* (1935). These investigators found that the red peel of Bramley's Seedling apples contained twice as much vitamin C as the green peel. Although they did not mention light as a factor in the production of this difference in vitamin content, it is well known that the red side of an apple is normally the one that has been exposed on the tree to direct sunlight.

A review of the literature in 1936 failed to show that a comparison as to vitamin C content had been made between citrus fruits from shaded and exposed parts of the tree. It had long been common knowledge, of course, that shaded fruit degreens later than exposed fruit and sometimes never completely degreens, and that its juice quality as judged by the taste test is not generally so high.

In 1936 an investigation was begun primarily to determine whether insolation influences the vitamin C content of Florida-grown citrus fruits; total soluble solids and total acid also were measured. Results of this study, terminated in 1943, are reported herein.

Harding, Winston, and Fisher (1939 and 1940) reported analyses indicating that Valencia and Lue Gim Gong oranges exposed to direct sunlight on the tree contained significantly more vitamin C than those not so exposed. Harding and Thomas (1942) reported that grapefruit obtained from the outside branches of the tree contained a little more vitamin C than that obtained from the inside branches.

MATERIALS AND METHODS

In December, 1936, initial tests were made to determine the vitamin C content, total acid, and total soluble solids of Dancy tangerines (*Citrus reticulata* Blanco). Later, Temple oranges (supposedly *C. reticulata* x *C. sinensis*) and early, midseason, and late varieties of round oranges [*C. sinensis* (L.) Osbeck] were included in the study. Between 1936 and 1943 juice of 44 lots of round oranges from widely separated groves of the varieties Parson Brown, Hamlin, Pineapple, Indian River, Seedling, and Valencia; of seven lots of Temple oranges; and of 11 lots of Dancy tangerines from groves in central Florida were analyzed. The Temple oranges were budded on rough lemon [*C. limon* (L.) Burm. f.] or sour orange (*C. aurantium* L.).

Like numbers of exposed fruits and of shaded fruits were taken from the same trees. Each test sample consisted of the composited juice of 25

TABLE 1

Influence of Insolation on Total Soluble Solids, Total Acid, and Vitamin C (Ascorbic Acid) Content of the Juice of Florida-Grown Round Oranges

Variey and date of test	Fruits tested		Total soluble solids		Total acid		Vitamin C Content	
	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits
	No.	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Mg./ml.*	*Mg./ml.*
Parson Brown								
Nov. 10, 1938	50	52	11.33	9.53	1.284	1.190	.62	.50
Nov. 4, 1940	50	50	10.15	9.25	1.042	1.340	.62	.48
Nov. 15, 1940	50	50	10.60	9.33	.944	1.126	.53	.40
Nov. 15, 1940	50	50	11.13	10.43	1.346	1.230	.61	.49
Average	50	50.5	10.80	9.64	1.154	1.222	.60	.47
Hamlin								
Nov. 4, 1940	50	50	9.84	8.98	.940	.990	.57	.49
Nov. 15, 1940	50	50	8.93	8.43	.904	.948	.54	.45
Average	50	50	9.39	8.71	.922	.969	.56	.47
Pineapple								
Dec. 17, 1937	55	51	11.33	9.53	1.284	1.190	.62	.50
Dec. 17, 1937	45	44	9.98	8.73	1.190	1.280	.53	.45
Dec. 21, 1937	46	48	9.71	7.97	1.002	.990	.49	.41
Dec. 21, 1937	51	50	9.46	8.91	1.158	1.054	.58	.50
Dec. 21, 1937	59	51	10.66	9.23	1.434	1.422	.63	.55
Dec. 21, 1937	52	51	10.66	9.20	1.430	1.422	.64	.55
Jan. 20, 1938	55	50	9.89	8.90	1.018	.984	.43	.34
Jan. 20, 1938	52	53	9.50	8.10	.604	.535	.34	.28
Jan. 20, 1938	50	50	11.79	9.84	1.130	.984	.48	.38
Jan. 20, 1938	50	50	10.59	9.70	.981	1.022	.47	.39
Feb. 2, 1938	50	50	11.66	11.26	1.062	1.062	.59	.57
Feb. 2, 1938	50	50	11.71	11.06	.928	.944	.57	.51
Feb. 2, 1938	50	50	12.31	11.91	.978	.972	.56	.51
Feb. 2, 1938	50	50	11.73	11.13	.872	.888	.56	.48
Feb. 2, 1938	50	50	12.98	12.38	1.010	1.002	.63	.56
Feb. 2, 1938	50	50	12.83	12.38	.900	.892	.53	.48
Nov. 20, 1940	50	50	11.62	10.62	1.266	1.324	.69	.52
Average	50.9	49.9	11.08	10.05	1.073	1.057	.55	.47
Indian River								
Dec. 21, 1937	49	50	10.03	9.53	1.613	1.414	.51	.42
Dec. 21, 1937	56	50	10.58	9.23	1.142	1.268	.39	.35
Dec. 21, 1937	51	55	11.43	9.73	1.716	1.750	.52	.38
Average	52	51.7	10.68	9.50	1.490	1.477	.47	.38
Seedling								
Jan. 9, 1937	25	25	12.84	11.86	1.466	1.570	.62	.52
Jan. 9, 1937	50	25	11.71	10.64	1.199	1.316	.62	.50
Jan. 23, 1937	25	25	12.53	11.43	1.138	1.272	.59	.50
Feb. 2, 1937	25	25	12.64	12.53	1.162	1.193	.59	.49
Average	31.3	25	12.43	11.62	1.241	1.338	.61	.50
Valencia								
Jan. 9, 1937	25	25	10.64	10.11	1.870	1.864	.60	.50
Jan. 16, 1937	25	25	10.21	8.11	1.217	1.126	.53	.36
Jan. 23, 1937	25	25	10.03	8.83	1.074	1.220	.50	.43
Feb. 13, 1937	25	25	10.50	9.70	1.094	1.142	.39	.32
Feb. 13, 1937	25	25	9.90	8.50	1.122	1.074	.45	.34
Feb. 20, 1937	26	26	10.34	9.24	1.054	1.086	.49	.37
Mar. 13, 1937	26	27	10.67	10.00	1.094	1.070	.42	.34
Mar. 13, 1937	31	26	10.90	8.67	1.022	1.002	.47	.36
Apr. 16, 1937	25	25	11.15	9.65	.960	.934	.38	.30
Apr. 16, 1937	27	28	11.50	8.75	.908	.824	.47	.34
May 12, 1937	28	31	11.80	9.73	.904	.764	.39	.28
May 12, 1937	36	34	11.73	8.09	.774	.649	.43	.30
May 22, 1937	12	12	12.26	9.71	.690	.607	.43	.37
June 4, 1937	24	24	9.16	8.65	.592	.582	.39	.37
Average	25.7	25.6	10.77	9.12	1.027	.996	.45	.36
Average for all varieties	41	40.1	10.98	9.76	1.103	1.103	.52	.43

to 52 representative fruits of average size from 10 to 15 trees. The methods used for determining total soluble solids, total acid, and vitamin C were identical with those described by Harding, Winston, and Fisher (1940), except that a Brix spindle was used to measure the total soluble solids.

RESULTS

Round Oranges. The exposed fruits of each of the six varieties of round oranges contained on the average larger percentages of total soluble solids than did comparable shaded ones (Table 1). For the 44 lots tested, regardless of variety, the average difference was 1.65 in percentage points, or 18.1 per cent, which is mathematically highly significant.

Statistical analysis of the data on total acid revealed no significant difference between exposed and shaded fruit.

Exposed oranges were consistently higher in vitamin C than shaded fruit. The tests on 44 lots showed that on the average the outside fruit contained 0.09 mg. per milliliter more vitamin C than the shaded fruit, a difference of 20.9 per cent. The results were highly significant statistically.

Temple Oranges. For the seven lots of Temple oranges analyzed, total solids were not significantly greater in the exposed than the shaded fruit. The difference amounted to 1.02 in percentage points, or 9.0 per cent (Table 2).

For four of the seven lots of Temple oranges, total acid was significantly higher in exposed fruit than in shaded fruit. On the average, the acid content of the exposed fruit was greater by 0.049 percentage point.

The vitamin C content of Temple oranges averaged 0.08 mg. per milliliter, or 16.7 per cent higher for the outside than for the inside samples. This difference was found to be statistically highly significant. As the samples were not collected to determine whether kind of rootstock had any effect, it is possible that they differed in other respects besides rootstock; but since Harding and Thomas (1942) reported that grapefruit had a higher ascorbic acid content (but the difference was not significant) when the trees were budded on rough lemon rootstock than when on sour orange, the reverse relation noted in this study is interesting.

Tangerines. In 11 lots of Dancy tangerines picked at various times and from several different groves during the 1936-37 harvest season, the outside (exposed) fruit consistently had more total soluble solids and more vitamin C than did the inside fruit, which was highly significant (Table 2). There was less total acid in the outside fruit, and this difference was highly significant, though less marked than the differences in other constituents. On an average, the exposed fruit was 23 per cent higher in soluble solids, 27 per cent higher in vitamin C, and 16 per cent lower in total acid.

DISCUSSION

As oranges mature, normally there is an increase in total soluble solids and a decrease in acid. It seems logical to assume that oranges on the outside branches of the tree mature more rapidly than those on inside branches, since in the former the solids were found to be higher than in the latter. However, even after both types of fruit have attained full

maturity, there is a vast difference in quality between the two. It seems likely that, just as most of the higher plants require direct sunlight for best growth and development, exposed branches produce better oranges than shaded ones.

The higher vitamin C content of the exposed oranges is no doubt a definite result of the incidence of sunlight. Other investigators, Hamner

TABLE 2

Influence of Insolation on Total Soluble Solids, Total Acid, and Vitamin C (Ascorbic Acid) Content of the Juice of Florida-Grown Temple Oranges and Dancy Tangerines.

Date of test	Fruits tested		Total soluble solids		Total acid		Vitamin C Content	
	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits	Exposed fruits	Shaded fruits
TEMPLE ORANGES								
	No.	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Mg./ml.*	*Mg./ml.*
Dec. 21, 1937	52	50	10.53	9.22	1.450	1.482	.50	.44
Jan. 20, 1938	53	55	11.89	11.09	1.205	1.070	.53	.45
Feb. 11, 1943	50	50	13.29	11.99	1.458	1.340	.68	.58
Feb. 11, 1943	50	50	13.49	12.99	1.644	1.744	.58	.51
Average	51.3	51.3	12.30	11.32	1.439	1.409	.57	.50
Jan. 20, 1938	50	50	10.84	9.79	1.082	.928	.47	.38
Feb. 11, 1943	50	50	14.18	12.79	1.616	1.623	.59	.49
Feb. 11, 1943	50	50	12.19	11.39	1.344	1.268	58	.51
Average	50	50	12.40	11.32	1.347	1.273	.55	.46
DANCY TANGERINES								
Dec. 12, 1936	27	27	9.63	7.80	.912	1.086	.24	.16
Dec. 12, 1936	25	25	9.32	7.20	.751	.872	.26	.22
Dec. 12, 1936	25	25	9.40	6.12	.666	.662	.31	.20
Dec. 22, 1936	25	25	10.30	8.10	.924	1.228	.37	.33
Dec. 22, 1936	25	25	10.95	9.10	1.264	1.399	.44	.33
Dec. 22, 1936	25	25	10.60	8.90	1.395	1.432	.29	.24
Dec. 22, 1936	25	25	10.90	8.60	1.392	2.011	.35	.24
Jan. 9, 1937	25	25	10.61	8.21	.649	.908	.20	.18
Jan. 23, 1937	25	25	10.83	9.33	.710	.760	.20	.17
Feb. 6, 1937	25	25	11.29	9.75	.660	.714	.21	.16
Feb. 20, 1937	31	30	11.00	10.38	.662	.784	.20	.16
Average	25.7	25.6	10.44	8.50	.908	1.078	.28	.22

et al. (1945), Hamner and Parks (1944), Reid (1942), Veselkine *et al.* (1934a), and Ezell *et al.*, have reported instances in which it was evident that direct sunlight increased the vitamin C content of plants. Mention has already been made of the report of Zilva *et al.* (1935), that the red peel of apples contained more ascorbic acid than did the green. Ezell and his associates have shown that strawberries grown in the shade contained significantly less ascorbic acid than did those exposed to normal sunlight. Kohman and Porter (1940) found that tomato plants set out in flats lost vitamin C from stems and leaves when held in a laboratory in subdued light, but showed an increase in this vitamin when the flats were removed to the roof of the building.

In the past, citrus growers have been warned against planting orange trees too close to each other, attention being directed to the fact that shaded fruit does not attain maximum color even when mature. This observation is most strikingly true of tangerines and of Temple oranges early in the season, but is not so marked when the fruit attains full maturity. The results of the present investigation indicate an additional reason for comparatively wide spacing of citrus trees, that is, to prevent unnecessary shading of fruit with consequent inferiority in general juice quality and in vitamin C content. This holds for round oranges, tangerines, and Temple oranges. During recent years there has been a rapid increase in the amount of citrus fruit canned, both as hearts and as juice. Juice quality, rather than rind appearance, determines the market value of citrus offerings to canneries. It is becoming more and more economically important to produce fruit of high nutritive quality as well as of attractive external appearance.

SUMMARY

In these investigations vitamin C content was found to be significantly higher in fruit from outside branches than in those from inside branches of the same tree. This was true for all varieties of round oranges studied, which included Parson Brown, Hamlin, Pineapple, Indian River, Seedling, and Valencia, as well as for Temple oranges, and for Dancy tangerines. Percentage of total soluble solids was significantly higher in the exposed fruit of all varieties tested. Total acid averaged somewhat higher in the outside Temple fruit than in that from the inside branches, while Dancy tangerines showed the reverse difference. Round oranges, including early, midseason, and late varieties, showed no significant difference in total acidity between fruit collected from inside and outside branches.

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ASCORBIC ACID AND DEHYDROASCORBIC ACID CONTENT OF RAW AND COOKED VEGETABLES[1]

ISABEL NOBLE AND MARY MARGARET DEVORE HANIG
Division of Home Economics, University of Minnesota, St. Paul, Minnesota

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It has been generally assumed that the quantity of dehydroascorbic acid present in fresh raw and cooked vegetables is so small that no serious error in the true vitamin C values results if the assay is made by the indophenol method. This assumption was supported by the work of Bessey (1938) who, using the hydrogen sulfide method, found no dehydroascorbic acid in fresh cauliflower, spinach, or green beans; and by that of Roe and Oesterling (1944) who, using their phenylhydrazine method, found only four of the twelve market vegetables tested to contain dehydroascorbic acid in excess of ten per cent of the total ascorbic acid. Recently, however, this assumption has been questioned on the basis of results obtained with raw fruits and vegetables before and after cutting or chopping, Smith and Caldwell (1945) and McMillan and Todhunter (1946); and with cooked cabbage which had stood on a steam table for two hours before sampling McMillan and Todhunter (1946).

The present study was undertaken, therefore, to determine the quantity of dehydroascorbic acid present in a sampling of market vegetables before and after cooking, and to calculate from these results the size of the error that may be expected when such vegetables are analyzed for only reduced ascorbic acid.

EXPERIMENTAL PROCEDURE

Vegetables Used and Their Sources: Cabbage, cauliflower, spinach, rutabagas, peas, and asparagus were purchased from city markets. All these vegetables except the asparagus had been shipped to the markets from other states, but they were fresh in appearance. They were used soon after purchase and were held at approximately 5°C.(41°F.) during any period that did elapse. Neither the variety nor the details of handling before purchase were known.

Preparation of Samples: Each purchase lot of each vegetable was divided into as similar portions as possible for the raw analyses and for four methods of cooking. As little time as possible was allowed to elapse between the first cutting of the vegetable and the running of the analyses. Details of obtaining the samples were as follows:

(a) *Cabbage:* The heads of cabbage (at least eight for each lot) were cut through the heart into quarters. A small amount was quickly shredded from each quarter for the raw sample, then one quarter-section from each head was used in the four portions to be cooked. These portions were coarsely shredded and each one well mixed just before cooking.

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(b) *Cauliflower:* The heads of cauliflower (at least five for each lot) were separated into flowerets and divided into five similar portions according to the size, location, and general appearance of the flowerets. One portion was used for the raw sample and the others were cooked.

(c) *Spinach Leaves:* The spinach (one-half bushel for each lot) was washed and placed on towels where it was shaken gently and blotted lightly to remove the surface moisture without bruising the leaves or letting them stand for any length of time. The stems were removed at the base of the leaves and the lot divided into five portions, care being taken to obtain in each portion as similar distributions as possible of small and large leaves.

(d) *Rutabagas:* Rutabagas used were wax-covered and had been stored four to five months. Those in each lot (six to eight in number) were peeled, quartered, and the quarters distributed among four portions. Each portion was cut into three-quarter-inch cubes and mixed thoroughly. Several cubes from each of the four portions were mixed together and used as the raw sample.

(e) *Peas:* The peas in each lot (about one-half bushel) were shelled, well-mixed, and divided into five portions.

(f) *Asparagus:* The woody ends were removed from the stalks in each lot (about ten pounds). The stalks were washed, blotted dry, and divided into five portions, care being taken to have an equal number of small, medium, and large spears in each portion. The small spears were cooked whole, while the stems of the larger ones were slit lengthwise into quarters or halves, to within one inch of the tip.

Cooking of Samples: A 25- or 30-gram portion, depending upon the expected ascorbic acid potency, of each mixed vegetable sample was weighed immediately after preparation and tied in a loose cheesecloth bag. These portions, together with sufficient additional vegetables from the sample to yield a four-serving portion as defined by Halliday and Noble (1946), were cooked by one of the following methods: Above the water level in a pressure saucepan at 15 pounds pressure; in a tightly covered pan in only enough water to prevent scorching; steaming; and in sufficient boiling water to cover during the entire cooking period (referred to later as the open kettle method). Quantities of water were the same as recommended by Halliday and Noble (1943). The cooking times were the minimum periods during which the vegetables would reach the tender stage. They were determined for each lot by preliminary cookings and were as follows for the pressure saucepan, tightly covered kettle, steamer, and boiling water: cabbage, 1.5-2.0, 17-18, 14-15, 8.5-9.0; cauliflower, 1.5, 26, 18-19, 8.0-8.5; spinach leaves, 0.5, 13, 9-10, 4.5-5.0; rutabagas, 4.0, 55-56, 35, 30; peas, 1.0, 32, 28, 17; asparagus, 1.0-1.5, 25, 22-23, 9-10 minutes. At the end of the cooking period, the vegetables were drained through cheesecloth for three to four minutes, the cooking waters set in an ice-bath to cool quickly, the bagged samples transferred to a Waring Blendor, and extracted as explained below.

Extraction of Samples: Twenty-five-gram raw portions (taken immediately after preparation of the sample) and the cooked samples from the bags were blended with 100-milliliter portions of three per cent meta-

phosphoric acid in a Waring Blendor, the suspension centrifuged, and aliquots of the clear supernatant liquid taken for ascorbic and dehydroascorbic acid determinations.

Ascorbic and Dehydroascorbic Acid Determinations: Ascorbic acid was determined on the vegetable extracts and on all of the cooking waters by the Morell method (1941), except that the correction of Bessey (1938) for color and turbidity was retained.

Dehydroascorbic acid determinations were made on the vegetable extracts and on the cooking waters from the tightly covered and the boiling water methods, by the Roe and Oesterling (1944) dinitrophenylhydrazine method. In preparing the vegetable extracts and cooking waters for these determinations, aliquots were mixed with sufficient metaphosphoric acid-thiourea solution to give the desired dilution. The concentration of the metaphosphoric-thiourea solution varied according to the size of the aliquot, but was such that the final concentration was three per cent metaphosphoric acid and one per cent thiourea. The method yielded good recoveries for all of the vegetables when known amounts of dehydroascorbic acid were added to extracts of raw samples. The average recovery was 100 per cent, with a range from 97 to 104 per cent.

Total Ascorbic Acid: Total ascorbic acid was calculated by adding the value obtained for ascorbic acid by the indophenol method to that obtained for the dehydroascorbic acid by the Roe and Oesterling method.

Moisture Determinations: Moisture was determined by loss in weight from three ten-gram portions from each raw sample during heating for 18 hours at 95°C.(203°F.) and 25 inches of vacuum.

Statistical Analysis: Minimum levels of significant difference between the means, for percentage retentions of ascorbic acid and dehydroascorbic acid in the cooked vegetables, were calculated as follows:

$$\text{Minimum level of significance at five per cent point} = t \times \sqrt{\frac{\text{Mean square for error} \times 2}{\text{Number of replications}}}$$

in which the mean square for error was taken from an analysis of variance in which cookings and replications were the sources of variation, and "t" equalled the "t" value at the five per cent point for the number of degrees of freedom of error.

DISCUSSION OF RESULTS

Ascorbic Acid Content of Raw Samples: Ascorbic acid content of the raw samples of the vegetables (Table 1) varied considerably from one purchase lot to another, especially for spinach leaves and rutabagas, for which the extreme figures differed from the mean by about 30 per cent. The variations were not greater, however, than those reported by other workers, Lampitt *et al.* (1943 and 1945), Roe and Oesterling (1944), and Adams and Smith (1944).

Retention of Ascorbic Acid During Cooking: The percentage of ascorbic acid retained in the cooked vegetables for any one of the cooking methods, like the ascorbic acid concentrations in the raw samples, showed variations from lot to lot. For example, figures for Lot 2 of cabbage (Table 2), when compared with only one another, would seem to indicate

the greatest retention in the sample cooked in the pressure saucepan, while those for Lot 3 would appear to show the highest retention in the sample cooked in the tightly covered pan. These variations might have been decreased to a certain extent by controlling the cooking procedures more rigidly, but the methods employed were as carefully controlled as would be possible under home conditions, which were the conditions of interest in this study. Therefore, in order to secure a reasonably reliable evaluation of the advantage, if any, which one method of cooking may possess over the others, all further comparisons have been based upon the means obtained from four replications and the minimum levels of significant differences at the five per cent level between these means.

TABLE 1

Ascorbic Acid Content of Raw and Cooked Vegetables

Vegetable	Lot No.	Moisture content of raw	Ascorbic acid content of raw	Ascorbic acid content of cooked (mg. per quantity equivalent to 100 g. raw fresh weight)			
				Pressure saucepan	Tightly covered kettle	Steamer	Open kettle
		pct.	*mg./ 100 gm.*				
Cabbage	1	86.6	49.8	36.7	35.3	37.6	14.1
	2	91.2	53.0	45.3	32.3	29.3	10.8
	3	87.9	73.7	49.3	57.9	50.9	24.1
	4	90.5	45.2	27.2	24.0	31.3	11.2
	Mean	.....	55.4	39.6	37.4	37.3	15.0
Cauliflower	1	88.6	63.1	45.4	30.3	43.1	23.8
	2	88.4	62.4	46.1	39.3	49.2	19.6
	3	90.0	39.9	30.6	22.5	28.2	17.2
	4	90.0	43.2	25.2	22.5	28.0	14.7
	Mean	.....	52.2	36.8	28.6	37.1	18.8
Spinach leaves	1a[1]	87.7	119.9	79.5	63.3	71.2	42.6
	1b[1]	88.0	91.7	69.4	58.7	68.1	45.6
	2	89.0	96.2	81.2	59.7	61.2	47.7
	3	90.1	53.2	46.3	39.0	30.7	22.3
	Mean	.....	90.2	69.1	55.2	57.8	39.6
Rutabagas	1	90.3	63.3	39.9	28.3	39.8	21.6
	2	90.7	26.8	18.6	15.9	16.3	9.7
	3	90.7	28.5	16.9	14.7	20.0	9.5
	4	90.6	25.7	18.1	20.0	17.3	10.7
	Mean	.....	36.1	23.4	19.7	23.4	12.9
Peas	1	95.9	28.7	16.1	17.3	18.5	13.1
	2a[1]	95.5	29.1	19.2	18.7	17.6	11.2
	2b[1]	95.3	26.0	15.3	18.6	18.1	12.7
	3	95.4	24.7	17.9	16.3	17.2	10.2
	Mean	.....	27.1	17.1	17.7	17.8	11.8
Asparagus	1	88.3	32.3	27.8	24.0	26.3	15.1
	2	87.5	29.2	26.8	21.0	24.1	10.2
	3	87.4	26.6	16.0	17.2	19.1	13.6
	4	89.9	28.1	22.6	20.4	21.4	10.5
	Mean	.....	29.0	23.3	20.6	22.7	12.4

[1] The letters "a" and "b" which follow the lot number indicate that samples were taken from the same day's purchase.

On this basis, some of the differences between means are not great enough to be significant, while others are. Thus, for cabbage, rutabagas, peas, and asparagus, the mean retentions of ascorbic acid were not significantly different in samples cooked in a pressure saucepan, tightly covered kettle, and steamer. They amounted to about two-thirds for cabbage, rutabagas, and peas, and about three-fourths for asparagus, of the quantity present in the raw sample. For cauliflower, however, cooking in a pressure saucepan or steamer gave significantly higher retentions of ascorbic acid than did cooking in a tightly covered pan; while for spinach leaves, cooking in a pressure saucepan gave significantly higher results than did any of the other methods. All of these methods (that is, cooking in a pressure saucepan, tightly covered pan, and steamer) gave

TABLE 2

Retention of Ascorbic Acid in Vegetables Cooked by Different Methods

Vegetable	Lot No.	Ascorbic acid retained in vegetable				Ascorbic acid dissolved in cooking water			
		Pressure saucepan	Tightly covered kettle	Steamer	Open kettle	Pressure saucepan	Tightly covered kettle	Steamer	Open kettle
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Cabbage	1	74	71	76	28	7	9	8	50
	2	85	61	55	20	8	6	5	35
	3	67	79	69	33	7	9	6	57
	4	60	53	69	25	7	9	6	60
	Mean[1]	72	66	67	26	7	8	6	50
Cauliflower	1	72	48	68	38	8	3	3	53
	2	74	63	79	32	5	2	4	64
	3	77	56	71	43	10	5	7	49
	4	58	52	65	34	6	3	3	57
	Mean[1]	70	55	71	37	7	4	4	56
Spinach leaves	1a	66	53	58	36	13	12	13	37
	1b	76	64	74	50	17	12	13	43
	2	84	62	64	50	15	15	12	39
	3	87	73	58	42	15	15	13	39
	Mean[1]	78	63	64	44	15	13	13	39
Rutabagas	1	62	45	63	34	7	11	10	42
	2	69	59	61	36	3	3	4	36
	3	59	52	70	33	8	8	9	35
	4	70	78	67	42	7	6	8	42
	Mean[1]	65	58	65	36	6	7	8	39
Peas	1	56	60	65	46	11	8	11	35
	2a	66	65	61	38	13	9	14	41
	2b	59	72	70	49	9	6	14	36
	3	73	66	70	41	11	6	16	40
	Mean[1]	64	66	66	43	11	7	14	38
Asparagus	1	86	74	81	47	3	7	5	12
	2	92	72	82	35	8	10	6	22
	3	60	65	72	51	9	8	7	17
	4	81	73	76	38	12	12	13	31
	Mean[1]	80	71	78	43	8	9	8	20

[1] The minimum level of significance between the means at the five per cent level equalled 14, 11, 10, 10, 9, and 13 for cabbage, cauliflower, spinach, rutabagas, peas, and asparagus, respectively.

significantly higher retentions than did cooking in enough boiling water to cover the samples, which method retained about one-fourth for cabbage and two-fifths for the other vegetables, of the ascorbic acid present in the raw samples.

Cooking waters from the pressure saucepan, tightly covered pan, and steamer contained less than 10 per cent of the amount of ascorbic acid in the raw sample when cabbage, cauliflower, rutabagas, and asparagus were cooked, and between 10 and 15 per cent when peas and spinach were cooked (Table 2). Those from the open kettle method contained around half the amount present in the raw sample when cabbage and cauliflower were cooked, about two-fifths when spinach leaves, rutabagas, and peas were cooked, and one-fifth when asparagus was cooked.

TABLE 3

Dehydroascorbic Acid Content of Raw and Cooked Vegetables

Vegetable	Lot No.[1]	Dehydroascorbic acid content of raw	Dehydroascorbic acid content of cooked (mg. per quantity equivalent to 100 g. raw fresh weight)			
			Pressure saucepan	Tightly covered kettle	Steamer	Open kettle
		mg./ 100 gm.				
Cabbage	1	4.7	3.6	3.2	3.6	1.4
	2	4.9	4.2	3.6	3.3	1.5
	3	7.6	5.4	5.6	5.8	1.4
	4	3.8	2.2	2.8	3.0	1.1
	Mean	5.2	3.8	3.8	3.9	1.4
Cauliflower	1	6.6	2.1	5.4	5.2	0.9
	2	6.1	1.7	3.9	4.8	1.2
	3	15.3	6.4	10.4	9.4	5.4
	4	4.6	1.4	3.1	2.9	1.2
	Mean	8.2	2.9	5.7	5.6	2.2
Spinach leaves	1a	7.6	6.3	6.4	3.9	4.8
	1b	7.7	5.0	6.2	2.9	5.1
	2	6.6	5.5	6.1	2.1	4.1
	3	4.7	2.6	3.4	2.6	3.3
	Mean	6.6	4.8	5.5	2.9	4.3
Rutabagas	1	3.6	2.0	2.2	1.5	1.5
	2	2.5	1.1	1.2	0.8	0.9
	3	2.4	1.4	1.2	0.5	1.0
	4	3.1	1.8	1.8	0.5	1.4
	Mean	2.9	1.6	1.6	0.8	1.2
Peas	1	2.1	0.7	1.4	1.5	0.5
	2a	1.8	0.7	0.9	1.3	0.5
	2b	3.0	1.0	2.2	2.0	0.8
	3	4.0	1.5	2.7	2.2	1.2
	Mean	2.7	1.0	1.8	1.8	0.8
Asparagus	1	2.6	1.0	1.8	1.4	0.4
	2	2.6	1.0	1.5	1.3	0.6
	3	2.6	0.9	1.8	1.6	0.6
	4	2.2	0.7	1.8	1.1	0.5
	Mean	2.5	0.9	1.7	1.4	0.5

[1] The letters "a" and "b" which follow the lot number indicate that samples were taken from the same day's purchase.

The amount of ascorbic acid found in the vegetable samples, plus their corresponding cooking waters, was about three-fourths the quantity present in the raw samples. Thus, about one-fourth of the original amount was not accounted for.

Dehydroascorbic Acid Content of Raw Samples: The dehydroascorbic acid content of all the vegetables was small: less than three milligrams per 100 grams for rutabagas, peas, and asparagus, and about five, seven, and eight for cabbage, spinach leaves, and cauliflower, respectively (Table 3). These figures are only 10 per cent (or less) of those for ascorbic acid. The ones for cauliflower, peas, and asparagus are very little higher than the values of Roe and Oesterling (1944), and that for cabbage (although the cabbage was coarsely shredded) is only slightly higher than the figure

TABLE 4

Retention of Dehydroascorbic Acid in Vegetables Cooked by Different Methods

Vegetable	Lot No.	Dehydroascorbic acid retained in vegetable				Dehydroascorbic acid dissolved in cooking water	
		Pressure saucepan	Tightly covered kettle	Steamer	Open kettle	Tightly covered kettle	Open kettle
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Cabbage	1	76	67	77	29	1	19
	2	86	74	69	32	3	27
	3	71	73	77	19	3	26
	4	57	74	78	29	6	10
	Mean[1]	72	72	75	27	3	20
Cauliflower	1	33	82	80	15	3	11
	2	28	64	78	21	1	13
	3	42	66	61	36	2	21
	4	32	66	64	28	4	40
	Mean[1]	34	70	71	25	3	21
Spinach leaves	1a	83	84	52	64	15	23
	1b	65	81	38	66	10	24
	2	84	93	32	62	13	32
	3	57	73	56	72	18	36
	Mean[1]	72	83	44	66	14	29
Rutabagas	1	56	62	42	42	3	5
	2	45	48	34	35	6	19
	3	61	51	22	41	5	19
	4	57	59	18	46	3	14
	Mean[1]	55	55	29	41	4	14
Peas	1	36	66	70	24	3	11
	2a	40	54	76	31	5	14
	2b	36	71	66	27	5	21
	3	38	68	57	32	3	13
	Mean[1]	38	65	67	28	4	15
Asparagus	1	41	70	56	17	7	8
	2	38	57	52	26	9	16
	3	35	67	61	25	7	18
	4	33	83	54	26	9	13
	Mean[1]	37	69	56	24	8	14

[1] The minimum level of significance between the means at the five per cent level equalled 12, 14, 17, 12, 11, and 11 for cabbage, cauliflower, spinach, rutabagas, peas, and asparagus, respectively.

reported by McMillan and Todhunter (1946) for cabbage wedges. It is half the amount reported by them for shredded cabbage.

The values found for the cooked vegetables (Table 3) were always less than those for the corresponding raw sample, thus showing that if any ascorbic acid was oxidized to dehydroascorbic acid during the cooking process, the oxidized form was as rapidly destroyed as the reduced form.

Retention of Dehydroascorbic Acid During Cooking: The percentage retention of dehydroascorbic acid in the vegetables followed no general pattern with regard to the various cooking methods (Table 4). Thus, the samples of cabbage cooked in a pressure saucepan, tightly covered kettle, or steamer showed retentions which were not significantly different from one another, the values being approximately three-fourths of the original amount. All three methods, however, gave significantly higher retentions

TABLE 5

Total Ascorbic Acid Content Present as Dehydroascorbic Acid in Raw and Cooked Vegetables

Vegetable	Lot No.	Raw	Cooked by different methods				Cooking water	
			Pressure saucepan	Tightly covered pan	Steamer	Open kettle	Tightly covered pan	Open kettle
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Cabbage	1	9	9	8	9	9	1	3
	2	8	8	10	10	12	5	7
	3	9	10	9	10	7	4	5
	4	8	7	10	9	9	6	13
	Mean	8	8	9	10	9	4	7
Cauliflower	1	9	4	15	11	4	5	2
	2	9	4	9	9	6	2	2
	3	28	17	32	25	24	7	14
	4	10	5	12	9	8	7	7
	Mean	14	8	17	14	10	5	6
Spinach leaves	1a	6	7	9	5	10	7	4
	1b	8	7	10	4	10	6	4
	2	6	6	9	3	8	6	5
	3	8	5	8	8	13	10	8
	Mean	7	6	9	5	10	7	5
Rutabagas	1	5	5	7	4	6	2	1
	2	8	6	7	5	6	16	4
	3	8	8	8	2	10	5	4
	4	10	9	8	3	12	6	4
	Mean	8	7	8	4	8	7	3
Peas	1	7	4	7	8	4	2	2
	2a	6	4	5	7	4	3	2
	2b	10	5	10	10	6	8	6
	3	14	8	14	11	10	7	5
	Mean	9	5	9	9	6	5	4
Asparagus	1	7	3	7	5	2	7	5
	2	8	4	7	5	6	7	6
	3	9	5	9	8	4	7	9
	4	7	3	8	5	4	7	5
	Mean	8	4	8	6	4	7	6

than samples cooked in an open kettle, which retained only one-fourth of the original amount. On the other hand, cauliflower and peas cooked in a tightly covered pan and steamer showed no significant difference, retentions being a little less than three-fourths and about two-thirds, respectively, of the original amounts. Both methods gave significantly higher retentions than did a pressure saucepan or open kettle, which were not significantly different from one another and retained about one-third of the original amount. Spinach leaves, rutabagas, and asparagus gave still different arrangements when the four cooking methods were placed in descending order for percentage retentions.

Cooking waters from the tightly covered kettle contained three to four per cent of the dehydroascorbic acid present in the raw sample for cabbage, cauliflower, rutabagas, and peas; eight and 14 per cent for asparagus

TABLE 6

Retention of Total Ascorbic Acid in Vegetables Cooked by Different Methods

Vegetable	Lot No.	Total ascorbic acid retained in vegetable				Total ascorbic acid dissolved in cooking water		Total ascorbic acid in vegetable plus cooking water	
		Pressure saucepan	Tightly covered pan	Steamer	Open kettle	Tightly covered pan	Open kettle	Tightly covered pan	Open kettle
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Cabbage	1	74	71	76	28	8	47	79	76
	2	85	62	56	21	6	34	68	56
	3	67	78	70	31	8	52	83	83
	4	60	54	70	24	9	56	62	81
	Mean	72	66	68	26	8	47	73	74
Cauliflower	1	68	51	69	35	6	49	57	84
	2	70	63	79	30	5	59	68	88
	3	67	60	68	41	8	41	67	82
	4	56	54	65	33	6	55	59	89
	Mean	65	57	70	35	6	51	63	86
Spinach leaves	1a	67	55	59	37	12	36	67	73
	1b	75	37	71	51	12	42	77	92
	2	84	64	62	50	15	38	79	89
	3	84	73	58	44	15	39	89	83
	Mean	77	57	62	46	14	39	78	84
Rutabagas	1	63	46	62	34	11	40	56	74
	2	67	58	58	52	3	40	82	91
	3	61	51	68	34	8	34	59	68
	4	67	76	60	42	6	39	81	81
	Mean	64	58	62	41	7	38	70	79
Peas	1	54	61	65	44	8	29	68	73
	2a	64	63	61	38	9	38	72	76
	2b	56	72	69	46	6	34	78	81
	3	68	66	68	40	6	36	72	76
	Mean	60	66	66	42	7	34	72	76
Asparagus	1	82	74	79	44	7	12	81	56
	2	87	71	80	34	10	21	81	55
	3	58	65	71	49	8	17	73	66
	4	77	73	74	36	9	20	82	56
	Mean	76	71	76	41	8	18	79	56

and spinach leaves, respectively. Those from the open kettle contained between 15 and 20 per cent of the original amount for all the vegetables, except for spinach leaves, which averaged 29 per cent.

The amount of dehydroascorbic acid found in the vegetable samples and cooking waters varied considerably from vegetable to vegetable for both the tightly covered pan and the open kettle. For example, it ranged from about half the amount originally present in the raw sample (rutabagas) to 95-97 per cent of that amount (spinach leaves). Thus, from 50 to about five per cent of the original dehydroascorbic acid was not recovered.

Proportion of Total Ascorbic Acid Found as Dehydroascorbic Acid: The average amount of dehydroascorbic acid present in the raw and cooked samples of each of the vegetables, except cauliflower, and in the cooking waters, was 10 per cent or less of the total ascorbic acid found in that sample (Table 5). The raw and two cooked portions of cauliflower averaged slightly higher than the other vegetables—around 15, rather than 10 per cent. Thus, it would appear that if only the (reduced) ascorbic acid was determined, the values usually would be within 10 per cent of the total ascorbic acid.

The Retention of Total Ascorbic Acid During Cooking: The percentage of total ascorbic acid retained in the vegetables and dissolved in the cooking waters was practically the same as that of the (reduced) ascorbic acid (Tables 6 and 2). The comparison of the various cooking methods made previously on the basis of the retention of ascorbic acid, therefore, is true also for the total ascorbic acid.

SUMMARY

Ascorbic and dehydroascorbic acid contents of four market lots of raw and cooked cabbage, cauliflower, spinach leaves, rutabagas, peas, and asparagus have been determined by the indophenol and dinitrophenylhydrazine methods, respectively.

According to the minimum levels of significant difference at the five per cent point, the mean retentions of ascorbic acid in cabbage, rutabagas, peas, and asparagus were not significantly different in samples cooked in a pressure saucepan, tightly covered kettle, and steamer. They amounted to about two-thirds for cabbage, rutabagas, and peas, and about three-fourths for asparagus, of the quantity present in the raw sample. For cauliflower, however, cooking in a pressure saucepan above the water level or steamer gave significantly higher retentions of ascorbic acid than did cooking in a tightly covered pan in the minimum amount of water to prevent scorching; while for spinach leaves, cooking in a pressure saucepan gave significantly higher results than did any of the other methods. All of these methods gave significantly higher retentions for all the vegetables than did cooking in enough boiling water to cover, which method retained about one-fourth for cabbage and two-fifths for the other vegetables of the ascorbic acid present in the raw samples.

According to the same criterion, the percentage retention of dehydroascorbic acid in the vegetable samples followed no general pattern with regard to the various cooking methods. The amounts recovered in the

vegetables and cooking waters, however, were always less than the amount in the corresponding raw sample; thus showing that if any ascorbic acid was oxidized, to dehydroascorbic acid during the cooking process, the oxidized form was as rapidly destroyed as the reduced form.

The average amount of dehydroascorbic acid present in the raw and cooked samples of each of the vegetables, except cauliflower, and in the cooking waters, was 10 per cent or less of the total ascorbic acid found in that sample. (Cauliflower averaged 15 per cent for the raw and for two of the cooked samples.)

The percentage of total ascorbic acid retained in the vegetables and dissolved in the cooking waters was practically the same as that of the reduced ascorbic acid.

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RETENTION OF B VITAMINS AFTER LARGE-SCALE COOKING OF MEAT. I. SUITABILITY OF LEFT AND RIGHT MUSCLES FOR RETENTION STUDIES

SYLVIA COVER, ESTHER M. DILSAVER, AND RENE M. HAYS
Texas Agricultural Experiment Station, College Station, Texas

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The location of raw samples for retention studies of vitamins in cooked meat is such an important factor in the accuracy of such studies that it deserves special attention. In the present study, paired one-muscle roasts were compared for their vitamin content. The tests were made in 1944 and were preliminary to a study of retention after roasting by different methods in large-scale cookery.

EXPERIMENTAL PROCEDURE

Individual muscles were selected which were large enough for roasts. Individual muscles were used out of consideration for the difference in vitamin content of different muscles within the same animal reported by Pyke (1940) and by Brady, Peterson, and Shaw (1944). The single muscles used as roasts were: longissimus dorsi (eye of rib), semimembranosus (inside or top round), and biceps femoris (outside or bottom round) of both beef and pork; and semitendinosus (eye of round) of beef only. These muscles were dissected in the manner described below.

Method of Dissection

Longissimus dorsi: The entire pork loin was purchased. The first cutting was made between the two vertebrae at the junction of which was attached the seventh rib, counting from the rear. (The number of ribs in hog carcasses is not constant.) This cutting crowded the front edge of the seventh rib. The rear portion was then cut so that 11 vertebrae were left in the part saved for roasting. In beef, the rib-loin was purchased as one piece. The first cutting was made between the two vertebrae at the junction of which was attached the third rib, counting from the rear. This cutting crowded the front edge of the third rib. The rear portion was then cut so that seven vertebrae were left in the part saved for roasting. The bones were removed. All muscles except longissimus dorsi were trimmed off and the fat trimmed to approximately equal thickness in the paired cuts. These roasts were approximately 14 inches long.

Semimembranosus: The entire ham or round was purchased; the aitchbone and shank were removed. As the ham or round lay on the table, the broad, thin gracilis lay under the fat covering and over much of the top surface. It was cut through very carefully so as not to slash the underlying muscles. The gracilis was then separated from the underlying muscles by cutting the connective tissue filaments. This exposed the semimembranosus which was the largest muscle then in view. It was removed intact, avoiding cuts and slashes.

Semitendinosus. After removal of the semimembranosus the semitendinosus was exposed as a long slender muscle near one edge. In pork it was too small to be used, but in beef it was removed intact.

Biceps femoris: This was the large muscle at the side of and nearer the bone than the semitendinosus. A depression in one side followed the shape of the semitendinosus. The thick upper end was cut when the pelvic bone was removed. The lower end was thin and joined to the connective tissue at the knee joint and over the muscles below. After dissection the shape of this muscle was quite irregular, with a narrow, thick strip down the middle tapering sharply to thin edges.

A fat covering of suitable thickness was left on these one-muscle roasts wherever possible. The thin flat ends and edges of the muscles from the round and ham were mainly connective tissue and were trimmed off to make the cuts more compact. Equal trimming was done in each roast of a pair. The weights of the single muscle roasts varied from 1.3 to 3.3 pounds in pork and from 1.7 to 5.4 pounds in beef.

Vitamin Methods

Thiamin was determined by the thiochrome method; pantothenic acid, riboflavin, and niacin by microbiological methods as described by Cover, McLaren, and Pearson (1944).

TABLE 1

Analyses of Paired Roasts of Beef, Both Raw

Cut of meat	Carcass No.	Vitamin content							
		Thiamin		Pantothenic acid		Niacin		Riboflavin	
		Left	Right	Left	Right	Left	Right	Left	Right
		Moist basis							
		μg/gm.	μg/gm.	μg/gm.	μg/gm.	μg/gm.	μg/gm.	μg/gm.	μg/gm.
Semimembranosus	I	1.91	1.91	2.92	2.97	43.6	45.5	1.38	1.45
	III	1.83	1.83	2.78	2.96	69.9	64.6	1.91	1.99
Biceps femoris	I	1.63	1.72	3.55	3.64	48.2	46.2	1.51	1.55
	III	2.06	2.12	3.76	3.83	56.4	55.3	1.43	1.41
Longissimus dorsi	II	1.39	1.38	2.49	2.32	71.1	69.8	1.35	1.39
	III	1.56	1.56	2.47	2.45	65.9	61.0	1.46	1.43
Semitendinosus	I	1.77	1.76	3.08	2.90	43.2	45.6	1.34	1.33
	III	1.57	1.56	2.31	2.26	54.9	54.9	1.67	1.62
Mean		1.72		2.92		56.0		1.51	
		Dry fat-free basis							
Semimembranosus	I	8.73	8.35	13.34	12.98	202.9	207.1	6.31	6.34
	III	7.50	7.76	11.40	12.55	286.6	273.8	7.83	8.44
Biceps femoris	I	7.64	8.05	16.22	17.51	225.9	216.2	7.08	7.25
	III	8.84	8.84	16.13	15.97	241.8	230.8	6.13	5.88
Longissimus dorsi	II	6.46	6.31	11.58	10.60	330.4	319.0	6.28	6.35
	III	6.93	6.99	10.97	10.98	292.4	273.3	2.48	6.41
Semitendinosus	I	8.48	8.48	14.75	13.98	206.7	219.8	6.42	6.41
	III	6.66	6.90	9.79	9.99	233.0	242.7	7.08	7.16
Mean		7.68		13.05		250.2		6.74	

TABLE 2

Analyses of Paired Roasts of Pork, Both Raw

Cut of meat	Carcass No.	Vitamin content							
		Thiamin		Pantothenic acid		Niacin		Riboflavin	
		Left	Right	Left	Right	Left	Right	Left	Right
Moist basis									
		μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Semimembranosus	I	1.97	1.97	4.09	4.26	47.1	46.0	1.72	1.79
	II	19.34	19.05	6.78	7.06	58.6	61.5	2.37	2.42
	III	3.94	3.68	4.94	5.13	56.3	60.4	2.05	1.99
	IV	2.48	2.28	5.14	4.78	69.5	62.7	1.44	1.48
Biceps femoris	II	9.45	9.69	4.39	4.01	.....	.....	1.86	1.92
	III	2.63	2.65	4.24	4.56	40.0	35.3	1.47	1.42
	IV	1.52	1.43	4.26	4.36	54.8	54.4	2.01	2.06
Longissimus dorsi	V	10.62	10.81	5.21	4.82	62.9	60.3	1.23	1.15
	VI	1.72	1.72	3.16	2.79	69.3	66.1	1.20	1.17
	VII	2.30	2.41	4.69	4.54	74.6	71.7	1.19	1.16
Mean.....		5.58		4.66		58.4		1.66	
Dry fat-free basis									
Semimembranosus	I	9.52	9.38	19.75	20.75	227.6	218.7	8.31	8.52
	II	94.77	96.77	32.88	33.46	284.3	291.4	11.61	12.29
	III	18.68	16.96	23.42	23.65	266.7	278.6	9.72	9.17
	IV	10.89	10.08	22.56	21.13	305.2	277.1	6.32	6.54
Biceps femoris	II	74.37	75.10	32.53	30.87	.....	.....	14.64	14.88
	III	16.86	17.68	28.83	30.00	256.3	235.7	9.67	9.66
	IV	8.04	7.61	22.54	23.20	289.7	289.6	10.63	10.96
Longissimus dorsi	V	60.32	65.08	29.59	29.02	357.1	362.9	6.99	6.92
	VI	8.82	9.05	16.21	14.68	355.4	347.7	6.16	6.15
	VII	12.63	13.50	25.75	25.42	409.4	401.2	6.53	6.50
Mean.....		31.81		25.31		303.0		9.11	

RESULTS

The vitamin content on both the moist and the dry fat-free basis is given for beef (Table 1) and pork (Table 2).

To test whether the left roast was significantly different from its right pair-mate, an analysis of variance was made for these paired data. The F values were calculated from the actual content on the moist basis and also on the dry, fat-free basis. Significance at the five per cent level required an F value of 5.59 for beef and 5.12 for pork, but in no case was the F value for any vitamin above 1.99. It is obvious that no statistical significance can be attached to the small differences observed.

Hence, paired single-muscle roasts may be used with confidence for retention studies.

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EFFECT ON THIAMIN RETENTION OF ADDING A CARBOHYDRATE VEGETABLE TO BEEF STEW[1]

SYLVIA COVER AND WILLIAM H. SMITH
Texas Agricultural Experiment Station,
College Station, Texas

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The addition of carbohydrate-rich materials, such as cereals, has been shown by Rice *et al.* (1944) and Nymon and Gortner (1947) to stabilize the thiamin in dehydrated meat mixtures. The thiamin in certain carbohydrate vegetables, such as potatoes, appears to be rather stable during cooking, according to Fenton (1945). These observations suggest the possibility that the retention of thiamin in meat-carbohydrate vegetable mixtures, such as stews, may be greater than the sum of the retentions obtained from the foods cooked separately. Adding potatoes to meat stews is a common household practice. It was not known how this practice affects thiamin retention. An experiment designed to test this possibility is described.

EXPERIMENTAL PROCEDURE

The variables in this study were: Meat, raw and cooked; potatoes, raw and cooked; raw mixture and cooked mixture.

Meat for Stews

The beef carcasses weighed approximately 400 to 500 pounds. They were obtained from two sources, a packing house and the college abattoir.

The meat was taken from the brisket and cut into pieces approximately one inch square. Enough of the fat was removed to make all the meat edible. The pieces were thoroughly mixed and three and one-half pounds of the mixture placed in each package. The packages were placed in shallow pans, one layer deep, frozen, and kept in the deep freezer until needed. One package at a time was removed and placed in the laboratory refrigerator to defrost before cooking. Just before cooking, the contents of the package were removed, mixed well, and five samples of approximately 300 grams each were weighed—two for separate raw samples, one for cooked meat, one for the raw mixture, and one for the cooked mixture.

Potatoes for Stews

Potatoes were purchased from a local grocery, selected for uniformity of shape and smoothness of surface. The first three experimental tests were made with a dark-skinned potato (sold as "Idaho Russet") which was long, smooth, and cylindrical, giving sections of rather uniform weight.[2] When sprouting made it impossible to use this variety any longer, "new" potatoes (sold as "California Burbank") were purchased which were ob-

[1] Supported in part by a grant from the National Livestock and Meat Board.

long, rather flat, and very light in skin color.[2] Sectioning with the flatter potato was more difficult and sections were not all of the same weight.

Each potato, after paring, was cut into eight radial sections (wedges) with the cut extending from stem to bud end. As they were prepared, the sections were dropped into a pan of cool water, a separate pan for each potato. After all had been prepared, and just before weighing, the sections were blotted with a cloth to remove excess moisture. Four potato samples of approximately 300 grams each were made, using a representative number of sections from each potato.

A set of four samples prepared in this way at one time was analyzed raw, to observe the difference between samples. The variations between these samples for both "old" and "new" potatoes were from one to three per cent of the lowest value. This is well within the limits of experimental error.

Four samples of potatoes were used in each replication—one raw, one cooked, one for the raw mixture, and one for the cooked mixture. The time intervals between use of the first and last potato samples were less than one-half hour. Before cooking, each potato section was cut into four pieces.

Standardized Cooking Procedure

Enough cooked stew to make four servings was selected as the desirable quantity for preparation. Preliminary tests showed that 300 grams each of meat and potatoes were needed.

Only one method of stewing was used in this study. The stew was cooked by a combination of boiling and pressure at 15 pounds. Boiling water was added to the sample of raw meat in the pressure saucepan. It was cooked for 17 minutes at 15 pounds pressure. The pressure was brought to zero quickly. The broth containing the meat was returned to boiling as quickly as possible and the potatoes added. Then the mixture was boiled until the potatoes were tender by the fork test (15 minutes). The meat samples cooked alone were cooked in the same way, except that the potatoes were left out. The potato samples cooked alone were dropped into boiling water. After the liquid reached boiling again, it was kept boiling for 15 minutes. Numerous previous trials showed that, with this combination of times and temperatures, both the meat and potatoes were tender.

Each cooked product, including the cooking liquid, was transferred to a tared pan and the saucepan rinsed with 50 ml. water. The pan was covered with a wax-paper cap and set in cool water. When the contents were cool, the outside of the pan was dried, the cover removed, and the pan with its contents weighed.

Sub-Sampling for Analysis

The 300-gram samples of raw food were sub-sampled before blending, but the entire cooked sample was blended. The liquid used to facilitate blending was 0.04 N H_2SO_4.

[2] The description and picture given by Clark and Lombard (1946) for the tuber of "Russet Burbank" fit the dark-skinned potato. Their description for the tuber of "Burbank" fits the light-skinned potato.

Raw Foods: A 300-gram sample of raw meat was ground and mixed thoroughly, using both the coarse and fine plates of the household meat grinder. A 300-gram sample of raw potatoes was cut into very fine pieces with a sharp knife and thoroughly mixed. The separate blends of these foods contained exactly 100 grams of the food per 400 grams total blend. The blend mixture contained exactly 100 grams each of meat and potatoes, per 700 grams total blend.

Cooked Foods: The cooked meat was separated from its broth and ground through the coarse plate of the meat grinder and every crumb of meat removed with the hot acid from a wash bottle. The meat, its broth, and washings were combined, weighed, and transferred quantitatively to a blendor jar. The cooked potatoes and their cooking liquid were weighed and transferred quantitatively to a blendor jar. The weight in each blendor jar was adjusted with acid to approximately 700 grams. After blending, the contents were poured into a large bowl and sufficient acid added to secure the following concentrations of food: meat cooked alone, approximately 300 grams (raw basis) per 900 grams total blend; potatoes cooked alone, approximately 300 grams (raw basis) per 1,050 grams total blend; mixture, approximately 300 grams meat and 300 grams potato (both on raw basis) per 2,100 grams total blend.

Three samples of each blend were taken—two for determining thiamin content and one for recovery. Weighed aliquots were taken so that each of the three samples from each blend contained a total of approximately eight μg. of thiamin. The blended samples were weighed directly into 100-ml. volumetric flasks which had been tared with funnels in place. The funnel and neck of the flask were washed with 35 ml. of the acid.

Before the flasks were placed in the water bath, eight μg. of thiamin were added to each recovery sample. Unless good recoveries were obtained for each food, the entire test was discarded.

Thiamin Method

Preparation of Digest: Acid extraction was carried out in a boiling water bath for one hour. After cooling to below 50°C.(122°F.) NaAc (0.5 M) was added to pH 4.5, takadiastase (0.5 gram) was added to each flask, and the samples digested at 45°C.(113°F.) overnight.

After digestion, the flasks were cooled without shaking (to prevent foaming). Distilled water was added to bring the volume to 100 ml. After mixing thoroughly the digests were filtered through filter paper, discarding the first 10 ml. The rest was collected and stored in the refrigerator in glass-stoppered flasks until analyses could be run.

Base Exchange: The weight of the blend taken for digestion had been adjusted so that 20 ml. of the digest could be used on the column. (For the recovery samples only 10 ml. were used.) This allowed 1.5 to 2.5 μg. of thiamin on each column. The thiamin was removed from the column with hot 25 per cent KCl in 0.1 N HCl. The effluent was collected in 25-ml. volumetric flasks and made up to volume with 25 per cent KCl in water. After thorough mixing four ml. aliquots were used for thiochrome production, internal standard, and blank determinations.

Thiochrome Procedure: The assay procedure given in the Cereal Chemists' Bulletin (Vol. 2, 1942) was modified slightly. Sixty-ml. separatory funnels were used as oxidation chambers. Separation of the layers occurred on standing, instead of by centrifuging. Ethyl alcohol, instead of anhydrous sodium sulfate, was used for drying the thiochrome solution.

RESULTS

Thiamin values for the raw beef averaged 0.88 μg. per gram (Table 1). They were similar to those previously reported by Tucker, Hinman, and Halliday (1946) and by Cover, McLaren, and Pearson (1944), who likewise used the Hennessy technique. However, they were lower than the values reported by Cover and Dilsaver (1947) and by Cover, McLaren, and Pearson (1944) when the Harris technique was used, or by Booher and Hartzler (1939) who used biological assays.

TABLE 1

Thiamin Content and Retention Assay Values

Test number[1]	Thiamin content						Retention		
	Raw foods			Cooked stew					
	Meat	Potatoes	Mixture	Meat[2] alone	Potatoes[2] alone	Mixture[2]	Meat	Potatoes	Mixture
	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	pct.	pct.	pct.
1	0.81	1.20	1.11	0.40	1.11	0.81	49	93	73
2	0.91	1.17	1.05	0.57	1.09	0.77	63	93	73
3	0.97	1.34	1.07	0.48	1.24	0.86	49	93	80
4	0.92	0.85	0.91	0.49	0.77	0.68	53	91	75
5	0.90	0.93	0.88	0.46	0.80	0.71	51	86	81
6	0.82	0.89	0.88	0.41	0.81	0.65	50	91	74
7	0.84	0.83	0.84	0.37	0.74	0.61	44	89	73
8	0.87	0.92	0.87	0.42	0.90	0.72	48	98	83
Tests 1-3	0.90	1.24	1.08	0.48	1.15	0.81	54	93	75
Tests 4-8	0.87	0.88	0.88	0.43	0.80	0.67	49	91	77
All tests	0.88	1.02	0.95	0.45	0.93	0.73	51	92	77

[1]Tests numbered 1 to 3 were made in May, using "old" potatoes, probably Russet; tests numbered 4 to 8 were made in June, using "new" potatoes, probably Burbank. [2]The values for cooked stews, which included both solid and liquid portions, were calculated back to the raw moist weight of the foods used.

When the "old" potatoes were used, the raw potato values were always higher than those for the raw meat (Table 1). When the "new" potatoes were used, however, the values for raw potatoes and raw meat were quite similar. After cooking, the values for both "old" and "new" potatoes were higher than those for the meat. Thiamin values for raw potatoes averaged 1.24 μg. per gram for the "old" potatoes and 0.88 μg. per gram for the "new" potatoes. Values for raw potatoes have been reported in the literature which ranged from 0.53 μg. per gram by Heller, McCay, and Lyon (1943) to 1.86 μg. per gram by Booher and Hartzler (1939). Leichsenring reported values varying from 0.68 to 1.60 μg. per gram, associated with variety obtained in the National Cooperative Project on the

Nutritive Value of Potatoes. The varieties, Russet and Burbank, were not included in this report.

Retentions averaged 54 per cent for the meat cooked alone (Table 1). This is similar to the retentions of 52 and 55 per cent reported by Cover, Dilsaver, and Hays (1947) for beef and lamb stews, respectively; and 51 per cent by McIntire, Schweigert, and Elvehjem (1943) for veal and lamb stews.

With potatoes cooked alone, the retentions averaged 92 per cent (Table 1). Only a few studies on boiled potatoes have been reported in the literature. Aughey and Daniel (1940) reported retention of 80 per cent in paired whole potatoes boiled an average of 36 minutes. The thiamin in the cooking liquid was included in the analyses. Oser, Melnick, and Oser (1943) reported retentions of 96 per cent and 70 per cent in potatoes boiled by their "new improved" and their "old fashioned" methods, respectively. The main difference between their two methods was the quantity of cooking liquid, which apparently was discarded without analysis. None of these conditions of preparation corresponds with those used in the present study.

TABLE 2

Calculated Thiamin Content of Mixtures

Test number[1]	Raw mixture				Cooked mixture			
	Grams of raw food in mixture		Calculated thiamin content		Grams of raw food in mixture		Calculated thiamin content	
	Meat	Potatoes	Total μg.[2]	μg./gm.[3]	Meat	Potatoes	Total μg.[2]	μg./gm.[3]
1	100.0	107.4	209.9	1.01	300.0	322.2	477.6	0.77
2	100.0	100.0	208.0	1.04	300.0	300.0	498.0	0.83
3	100.7	100.1	231.8	1.15	302.0	300.2	517.2	0.86
4	100.0	100.0	177.0	0.89	300.0	299.9	377.9	0.63
5	100.0	101.2	184.1	0.92	300.0	303.5	380.8	0.63
6	100.1	101.2	172.2	0.86	300.4	303.6	369.1	0.61
7	100.0	100.3	167.2	0.83	300.0	300.9	333.7	0.56
8	100.3	101.2	180.4	0.90	300.9	303.6	399.6	0.66
Tests 1-3	100.2	102.5	216.6	1.07	300.7	307.5	497.6	0.82
Tests 4-8	100.1	100.8	176.2	0.88	300.3	302.3	372.3	0.62
All tests	100.2	101.4	191.3	0.95	300.4	304.2	419.2	0.69

[1] Tests numbered 1 to 3 were made in May, using "old" potatoes, probably Russet; tests numbered 4 to 8 were made in June, using "new" potatoes, probably Burbank. [2] Total content of entire cooked stew. Three times as much food was used in the cooked mixture as in the raw mixture. [3] The values for cooked stew, which included both solid and liquid portions, were calculated back to the raw moist weight of the foods used.

The main purpose of the present study was to observe whether the addition of potatoes to the meat caused an increase in retention above that for the two foods cooked alone. Thus, it was necessary to calculate the content of each mixture from the weight and thiamin content of each food assayed separately. These calculated values for the raw and cooked mixtures are given (Table 2). Only one-third as much food was used for the raw samples as for the cooked ones. The assayed values for thiamin content may be compared with the corresponding calculated values (Table 3).

All values were calculated back to the raw moist weight of the food. This was possible because the food and broth had been analyzed together as one

sample. For the raw mixture the means of the assayed and the calculated values were quite similar. In the cooked mixture the assayed values were only very slightly greater than those obtained by calculation. While the assayed values are not enough higher to be of practical importance in the diet, results seem to indicate a slight trend toward an increase in content owing to cooking the mixture.

Retentions based on the assayed values averaged 77 per cent, while those for the calculated values averaged 73 per cent (Table 3). The retentions based on assays for those mixtures in which the "new" potatoes were used were uniformly higher than those based on the calculated values, but none of the differences was very great. Apparently, assay of the mixture, instead of calculation on the basis of thiamin values of the individual foods, did not affect the percentage retention to a marked extent.

TABLE 3

Effect of Mixing on Thiamin Content and Retention

Test number[1]	Thiamin content				Retention in cooked mixture	
	Raw mixture		Cooked mixture		Based on assay of raw and cooked mixture	Based on calculated values for mixtures
	Assay[2]	Calculated[2]	Assay[2]	Calculated[2]		
	μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*pct.*	*pct.*
1	1.11	1.01	0.81	0.77	73	76
2	1.05	1.04	0.77	0.83	73	80
3	1.07	1.15	0.86	0.86	80	75
4	0.91	0.89	0.68	0.63	75	71
5	0.88	0.92	0.71	0.63	81	68
6	0.88	0.86	0.65	0.61	74	71
7	0.84	0.83	0.61	0.56	73	67
8	0.87	0.90	0.72	0.66	83	73
Tests 1-3	1.08	1.07	0.81	0.82	75	77
Tests 4-8	0.90	0.88	0.67	0.62	77	70
All Tests	0.96	0.95	0.73	0.69	77	73

[1]Tests numbered 1 to 3 were made in May, using "old" potatoes, probably Russet; tests numbered 4 to 8 were made in June, using "new" potatoes, probably Burbank. [2]The values for cooked stews, which included both solid and liquid portions, were calculated back to the raw moist weight of the foods used.

Potatoes cook much more quickly than meat. To prevent them from cooking to pieces, they were added, as usual, near the end of the cooking period. Perhaps this accounts for the small effect on retention when this carbohydrate vegetable is cooked with meat in a stew. To test this possibility other tests were made to obtain retentions after the pressure cooking period of 17 minutes and also after pressure cooking and boiling, as in the previous tests. Comparison of the results showed that the additional 15 minutes' boiling period did not destroy a measurable amount of additional thiamin. Thus, the household practice of cooking potatoes with beef as in a stew does not appear to increase thiamin retention to a practical extent.

There are other meat dishes in which the carbohydrate is added at an earlier point in the cooking of the mixture. Results from this study should

not be taken as conclusive proof that the thiamin content of all cooked mixtures may with impunity be calculated from the assay values of the individual foods.

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EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF CERTAIN MICROORGANISMS ASSOCIATED WITH FOOD SPOILAGE

DOROTHY CULLER[1] AND HARRY WEISER
Department of Bacteriology

AND

E. D. WITMAN
Department of Agricultural Chemistry,
The Ohio State University, Columbus, Ohio

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Since the isolation of the first "plant hormone," indole-acetic acid, in 1934, and the more recently isolated growth-regulators, many experiments have been completed to determine the effect of growth compounds on various plants. At the present time, however, there has been only one published report of the effects of 2,4-dichlorophenoxyacetic acid on bacteria and fungi.

Mitchell and Whitehead (1940) observed the effect of this material on the rate of ripening of detached fruit. The rate of starch hydrolysis in bean leaves when growth regulating substances were added to the plants was studied and results noted by Mitchell and March (1944). while Stevenson and Mitchell (1945) studied the bacteriostatic and bactericidal properties on *Aerobacter cloacae, Bacillus subtilis, Phytomonas tumefaciens,* and *Staphylococcus aureus.*

This work was undertaken to determine the effects of 2,4-dichlorophenoxyacetic acid on several selected Gram positive and Gram negative bacteria, a few species of fungi, and other plant pathogens, regarding the rate of spoilage of apples, pears, bananas, and oranges by these microorganisms when the fruits were treated with this compound. Additional studies included the influence of the growth compound on the rate of starch hydrolysis by bacteria, to demonstrate any increase in cellular division, to observe any physiological changes, and also to note the destruction time of the 2,4-d on bacteria.

METHODS AND MATERIALS

To determine the effect of 2,4-d on the growth rate of bacteria, *B. subtilis* and *S. aureus,* respectively, were inoculated into nutrient broth containing 500 and 1,000 p.p.m. of the growth regulator. At varying time intervals, one ml. of the culture was removed and dilution plates prepared. Nutrient agar controls were maintained and the plates incubated at 37°C. (98.6°F.) for 24 to 48 hours.

The effect of the growth compound on the rate of starch hydrolysis produced by *B. subtilis* was determined by growing the organism in a two per cent starch broth containing 100 p.p.m. of 2,4-d. The starch

[1]Federal Food Inspection Service, Detroit Union Produce Terminal, Detroit, Michigan.

broth was prepared by adding four ml. of starch solution to each flask containing 200 ml. of nutrient broth. The amount of unhydrolyzed starch remaining was measured by the Schaffer-Hartman quantitative method. Inoculated and uninoculated starch culture media, uninoculated nutrient media, and uninoculated starch 2,4-d media were used as controls and the tests run in triplicate.

The influence of 2,4-d on the rate of multiplication was determined by inoculating organisms into nutrient broth containing 500 and 1,000 p.p.m. of the growth-regulator. The rate of growth was measured by the increase in turbidity, recorded by the photo-electric colorimeter. Results were compared with the controls, which contained no 2,4-d.

Several bacteria were inoculated into nutrient broth containing 50,000 p.p.m. of the growth regulating compound. At one-minute intervals for the first 10 minutes, and five-minute intervals for the following 10 minutes, transfers were made onto nutrient agar slants. Destruction time was determined by observing the last tube showing growth.

Inhibiting effects of 2,4-d on selected Gram negative and Gram positive bacteria were determined by employing *Proteus vulgaris, Aerobacter aerogenes, Alcaligenes fecalis, Pseudomonas aeruginosa,* and *Serratia marcescens* for the Gram negative representatives, and the organisms *B. subtilis, B. mycoides, Sarcina lutea,* and *S. aureus* for the Gram positive group. Nutrient agar samples containing 500, 1,000, 2,000, 4,000, 5,000, 6,000, 8,000, 10,000, and 12,000 p.p.m. of the growth-regulator were prepared, streak and pour plates made, and the cultures incubated at 37°C. for five days.

Various bacteria were inoculated into gelatin, lactose, dextrose, sucrose, litmus milk, tryptone, nitrate broth, and lead acetate, each containing 2,4-d, to determine whether any physiological changes occurred after treatment with the growth compound.

Plant pathogens tested with varying concentrations of the growth regulator incorporated in nutrient agar were *Erwinia caratovora, Phytomonas tobaci, Phytomonas tubefaciens, Phytomonas faciens, Phytomonas phaseoli,* and *Erwinia phytophthora.* The plates were incubated at room temperature for from three to five days.

Collectotrichum phomoides, phytophthora cactorum, Glomerella cingulata, Phycomyces nitens, Penicillium expansum, Penicillium sp. (from soy bean seedlings), *Rhizopus nigricans, Scherotinia cinerea, Sclerotinia sclerotiorum, Helminthosporium turicum, Sclerotium rolfsii,* and *Sclerotium delphinii* were grown on potato dextrose agar to which 1,000, 5,000, 10,000, 20,000, and 40,000 p.p.m. of the 2,4-d were added. The cultures were incubated for 14 days at room temperature.

Unsprayed green oranges were obtained from Florida. Concentrations of 500, 250, 100, and 50 p.p.m. was prepared from the growth regulator, and different lots of the oranges completely immersed in the separate dilutions for five minutes; at the same time 0.5 ml. of the solution was placed in the stem cavity. The fruit was incubated at room temperature for 20 days.

The degree of ripeness of the individual fruit was determined by the United States Department of Agriculture standards employed in the grad-

ing of fruits. The orange color of the individual fruit was established as a percentage of their total surface area. The refractometer was used at the end of 20 days to determine the amount of total solids present and the results interpreted from the Chemical Handbook to give the total sugar present.

Uniform, unsprayed ripe oranges to which no color had been added were obtained from a local grocery. The fruits were treated in groups of three, with the growth regulator in concentrations of 500, 100, and 50 p.p.m. in the same manner as the green oranges, to determine the rate of spoilage of treated ripe fruit. All fruit was incubated at room temperature.

Bananas that were green and hard were obtained from a Columbus grocery and groups of six were treated with 100, 500, 1,000, and 2,000 p.p.m. of the 2,4-d Carbowax solution. A one per cent Carbowax solution was used as a carrier to distribute the compound on the fruit and act as a solvent for this compound. Controls were prepared and the fruit incubated at room temperature. Rate of ripening was determined by the color development and by the increase in softness of the tissue. Yellow color was estimated as a percentage of the total surface area, and spoilage by the percentage of darkened, soft tissue.

Unsprayed, healthy ripe apples and pears were obtained from a grocery in Columbus. Groups of five apples were submerged for three seconds in the 2,4-d Carbowax solutions in concentrations of 100, 500, 1,000, and 2,000 p.p.m. The apples were incubated at room temperature and observed daily until complete spoilage occurred. The fruit was checked for percentage of surface area containing infected or softened tissue.

EXPERIMENTAL WORK

The effect of 2,4-dichlorophenoxyacetic acid on the growth rate of bacteria is shown (Table 1). It appears that the growth of *B. subtilis* and *Staph. aureus* was affected by 2,4-d in the concentrations used. The

TABLE 1

Bacterial Counts of B. subtilis and S. aureus Grown in the Presence of Acetate Nutrient Broth Solution[1]

Number of hours	*B. subtilis*[2]	Control	*S. aureus*[3]	Control
0	6	4	1	2
2	2	1	4	23
4	7	24	8	1,105
6	4	150	10	3,900
8	10	305	8	12,000
20	28	340	6	19,600
32	59	672	3	22,500
44	96	1,130	3	25,900
56	130	1,460	2	36,300
68	112	1,310	5	12,000
70	152	2,740	1	19,100
82	170	3,490	2	23,800
94	120	3,590	0	22,000
106	67	2,420	1	36,000
118	48	2,100	1	39,000

[1] Each figure should be multiplied by 1,000. [2] 500 p.p.m. [3] 1,000 p.p.m.

non-spore-forming organism was greatly inhibited as compared to the spore-forming culture as shown (Table 1). The remaining content of starch of a two per cent starch 2,4-d nutrient broth solution in which *B. subtilis* was grown is given (Table 2). There was an inhibition of starch hydrolysis by *B. subtilis* after exposure to the 2,4-d. However, there was also a conversion of starch by the 2,4-d in the absence of any organisms; the inhibition of starch hydrolysis that was observed may have been due to the partial inhibition of the organisms by the growth regulator. The variation of starch in the uninoculated controls indicates the different amounts of starch in the flasks upon preparation.

TABLE 2

Starch Content of Starch 2,4-d-Nutrient Broth Solution in Which B. subtilis Was Grown

	Test 1	Test 2	Test 3
	mg.	*mg.*	*mg.*
Uninoculated broth	9.3	6.2	9.1
Inoculated starch broth	17.1	13.1	12.2
Uninoculated starch broth	38.2	32.8	28.6
Uninoculated 2,4-d starch broth (100 p.p.m.)	33.5	28.7	22.4
Inoculated 2,4-d starch broth (100 p.p.m.)	32.8	29.3	26.5

Aerobacter aerogenes, *B. subtilis*, and *S. lutea* were grown in 2,4-d and turbidity measured on a photoelectric colorimeter every two hours for the first eight hours and every 12 hours for the following five days. An increase in cell division would have been concluded if the turbidity readings of the organisms which were exposed to the growth regulator had increased at a faster rate than the reading of the control organism. However, no increase in the cellular division was noted by this method.

The destruction time of several bacteria grown in the presence of 50,000 p.p.m. of the growth-regulating substance is shown (Table 4).

TABLE 4

Destruction Time of Organisms Grown in the Presence of 50,000 p.p.m. of 2,4-d

Organism	Time
	min.
S. aureus	15
S. lutea	10
B. subtilis	30
E. coli	25
A. aerogenes	20
A. fecalis	20
S. marcesens	20

Gram positive organisms were found to be inhibited in lower concentrations of the growth regulator than the Gram negative organisms. *Ps. aeroginosa* was the most resistant of the tested organisms and *B. subtilis* one of the least resistant (see Table 5). No physiological changes were noted in any of the organisms tested in different culture media containing 2,4-dichlorophenoxyacetic acid. The pigmentation of *S. lutea*, *S. marcas-*

cens, and *S. aureus* was markedly increased in the presence of the 2,4-d; whereas the pigmentation of *Ps. aeroginosa* was lost in low concentrations of the 2,4-d.

TABLE 5

Concentration of 2,4-Dichlorophenoxyacetic Acid Inhibiting Growth of Several Gram Positive and Gram Negative Bacteria [1]

Organism	Concentration in p.p.m.									
	500	1,000	2,000	3,000	4,000	5,000	6,000	8,000	10,000	12,000
P. vulgaris	+	+	+	+	+	−	−	−	−	−
B. subtilis	+	+	−	−	−	−	−	−	−	−
A. aerogenes	+	+	+	+	+	+	+	+	+	+[3]
A. fecalis	+	+	+	+	+	+	+	−	−	−
B. mycoides	+	−	−	−	−	−	−	−	−	−
S. lutea	+	+	−	−	−	−	−	−	−	−
S. aureus	+	+	−	−	−	−	−	−	−	−
Ps. aeroginosa	+	+	+	+	+	+	+	+	+	+[4]
S. marcescens	+	+	+	+	+	+	+	+	+[2]	+

[1] + denotes growth; — denotes no growth. [2] Five days were required for growth of *S. marcescens* on agar containing these two concentrations. [3] Inhibited in 20,000 p.p.m. [4] Inhibited in 25,000 p.p.m.

Bacillus subtilis frequently produced an extremely rough colony when grown in the presence of other growth regulators. No marked colony changes were observed on the other test organisms used.

Plant pathogens belonging to the genus *Phytomonas* were inhibited in lower concentrations of the growth regulator than the *Erwinia* group. No physiological or morphological changes were noted. For concentration required to inhibit the growth of several plant pathogens see Table 6.

TABLE 6

Concentration of 2,4-Dichlorophenoxyacetic Acid Required to Inhibit Growth of Some Plant Pathogens

Organism	Concentration in p.p.m.									
	500	1,000	2,000	3,000	4,000	5,000	6,000	8,000	10,000	12,000
Er. caratovora	+	+	+	+	+	+	+	+	+	+
P. stewarti	+	−	−	−	−	−	−	−	−	−
P. tumefaciens	+	+	−	−	−	−	−	−	−	−
P. faciens	+	−	−	−	−	−	−	−	−	−
P. tobaci	+	−	−	−	−	−	−	−	−	−
P. phaseoli	+	+	−	−	−	−	−	−	−	−
Er. phytophthora	+	+	+	+	+	+	+	+	+	−

Few of the fungi tested were inhibited in concentrations of 10,000 p.p.m., and all the organisms were inhibited in concentrations of 40,000 p.p.m. Concentration of 2,4-d required to inhibit various fungi is given (Table 7).

In concentrations of 5,000 p.p.m., the growth regulator did not inhibit the growth of the fungi studied. However, concentrations of 40,000 p.p.m. completely inhibited the growth of the test organism.

The different concentrations of the growth regulator had no effect on the rate of ripening or on the rate of spoilage of oranges. Fourteen days after treatment both the treated and control oranges were ripe. Over-

TABLE 7

Concentration of 2,4-Dichlorophenoxyacetic Acid Required to Inhibit Growth of Several Fungi

Organism	Concentration in p.p.m.				
	1,000	5,000	10,000	20,000	40,000
C. cactorum	+	+	−	−	−
C. cingulata	+	+	−	−	−
D. nitens	+	+	+	+	−
P. expansum	+	+	+	+	−
R. nigricans	+	+	+	+	−
S. cinera	+	+	−	−	−
S. sclerotiorum	+	+	+	+	−
H. turcicum	+	+	+	−	−
S. relfsii	+	+	−	−	−
S. delphinei	+	+	−	−	−
P. sp. (seedlings)	+	+	+	+	−
C. phomoides	+	+	+	+	−

ripe oranges from each group were tested with the refractometer, to determine whether the sugar content had any effect on the rate of spoilage, and to find whether the total sugar present was altered after treatment with 2,4-dichlorophenoxyacetic acid. Results are given (Table 8). The ripe and green oranges inoculated with *Penicillium sp.* and treated with different concentrations showed no difference in the rate of spoilage.

TABLE 8

Total Sugar Present in Oranges Treated With 2,4-Dichlorophenoxyacetic Acid [1]

Test	500	250	100	50	Control
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
1	8.6	6.65	6.8	8.7	8.2
2	9.3	7.8	7.2	7.3	8.0
3	7.4	6.8	7.5	8.9	9.6
4	....	7.5	....	7.2	....

[1] Concentrations expressed in p.p.m.

The growth regulator influenced the rate of ripening and the rate of spoilage of bananas very markedly. Forty-eight hours after treatment the fruits immersed in a concentration of 2,000 p.p.m. of the acetate were ripe. Fruits ripened proportionally to the concentration of the growth compound used. An increase in the rate of ripening was followed by an increased rate of spoilage. Results are expressed (Tables 9 and 10).

TABLE 9

Rate of Ripening Bananas Held at Room Temperature After Treatment With 2,4-Dichlorophenoxyacetic Acid [1]

Test	100	500	1,000	2,000	Untreated control	Carbowax control
	days	*days*	*days*	*days*	*days*	*days*
1	5	3	3	2	6	6
2	5	5	3	2	6	6
3	6	5	3	2	6	6

[1] Concentration is in p.p.m.

TABLE 10

Rate of Spoilage of Treated Bananas Held at Room Temperature[1]

Test	100	500	1,000	2,000	Untreated control	Carbowax control
	days	*days*	*days*	*days*	*days*	*days*
1	4	6	7	5	10	12
2	8	7	6	6	6	6
3	9	9	5	5	9	10

[1] Concentration in p.p.m.

Effect of 2,4-d on the rate of spoilage of apples is expressed (Table 11). As indicated in that table, rate of spoilage was proportional to the concentration of the growth regulator used.

TABLE 11

Rate of Spoilage of Apples Treated With Concentrations of 100, 500, 1,000, and 2,000 p.p.m. of 2,4-Dichlorophenoxyacetic Acid and Incubated at Room Temperature

Test	100	500	1,000	2,000	Untreated control	Carbowax control
	days	*days*	*days*	*days*	*days*	*days*
1	37	8	7	6	20	40
2	23	12	7	6	17	25
3	14	15	6	7	18	20
4	18	13	5	4	15	23
5	9	17	9	8	19	30

Part of the surface area of the pears treated with the higher dilutions became darkened after the first day of incubation. After continued incubation this area became softened and was considered in the rate of spoilage. Results on the rate of spoilage are listed (Table 12).

TABLE 12

Rate of Spoilage of Pears Treated With Concentrations of 100, 500, 1,000, and 2,000 p.p.m. of 2,4-Dichlorophenoxyacetic Acid-Carbowax Solution and Incubated at Room Temperature

Test	100	500	1,000	2,000	Untreated control	Carbowax control
	days	*days*	*days*	*days*	*days*	*days*
1	6	35	22	4	20	38
2	15	5	4	4	25	27
3	15	10	6	5	4	24
4	18	15	10	9	5	21
5	7	17	12	15	19	18

DISCUSSION

The inhibiting effect of 2,4-dichlorophenoxyacetic acid on bacteria was directly proportional to the concentration of 2,4-d used. There was no change in the growth rate of the treated organism. The pattern of the growth followed the same growth curve as that of the untreated organism; however, the logarithmic growth phase was decreased and the maximum

stationary phase lengthened. No activation of cell division occurred and no change in cell morphology or physiology was noted in these experiments. In this manner, the action of the growth regulator on bacteria cannot be likened to the action on plants. Some of the physiological activities of plants are increased upon exposure to the regulator. If this increase was great enough, the death of the plant would seemingly result by "growing itself to death." No stimulation of starch hydrolysis occurred in *B. subtilis* treated with the growth compound, which seems to lead to the conclusion that metabolic processes were not enhanced and the death of the organism could not be explained in this manner.

The Gram positive organisms are inhibited in lower concentrations than the Gram negative bacteria, which suggests that this regulator could be used as a selective inhibitory agent.

Erwinia plant pathogens were destroyed only in too high a concentration to be practical for use as a bactericidal agent. Vegetables infected by these organisms would probably be harmed if the 2,4-d were used in concentrations great enough to kill these organisms. *Phytomonas* organisms were inhibited in low enough concentrations to assume a beneficial value in the use of this growth compound in preventing disease of this etiology. Concentrations affecting the growth of each plant and the bactericidal concentration of each plant pathogen must be determined before the complete beneficial value of this regulator can be determined.

None of the fungi was destroyed in concentrations low enough to consider the regulator a highly efficient fungicide in the treatment of plants.

It is perhaps only natural to assume that fruits treated in such a manner that the rate of ripening is accelerated would result, too, in an increase in the rate of spoilage. However, because of an increase in rate of physiological processes in this treatment, the spoilage would be due to tissue breakdown rather than to microbial growth. Rate of ripening or rate of spoilage could be controlled by varying the concentration of the regulator.

CONCLUSIONS

The growth curve pattern of *B. subtilis* and *S. aureus* did not change, but some of the phases were decreased or lengthened.

There was a decrease in the rate of starch hydrolysis produced by *Bacillus subtilis* in the presence of the growth regulator. (These results may have been due to the inhibition of the growth of this organism by the 2,4-d.)

Cellular division of *S. lutea, A. aerogenes,* and *B. subtilis* was not effected when grown in the presence of 2,4-d.

Destruction time of the *S. lutea* tested after exposure to 50,000 p.p.m. of the 2,4-d was 10 minutes, while *B. subtilis* required 30 minutes.

Gram positive bacteria were killed in lower concentrations than Gram negative.

There was no detection of any physiological changes of the bacteria tested due to 2,4-d.

Phytomonas plant pathogens were destroyed in lower concentrations than the *Erwinia* group.

Fungi tested were inhibited in comparatively high concentrations.

There was no effect on the rate of spoilage or on the rate of ripening of treated oranges.

Rate of ripening of bananas, and rate of spoilage and rate of ripening of both bananas and apples, were increased proportionally to the concentration of the growth regulator used. Rate of spoilage of treated apples was accelerated proportionally to the concentration of 2,4-d used. Spoilage of pears was enhanced by treatment with the growth regulator.

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INFLUENCE OF COOKING ON ASCORBIC ACID CONTENT OF COLLARDS[1,2,3]

HARRIET PRESSLY

Nutrition Section, Department of Animal Industry, North Carolina Agricultural Experiment Station, Raleigh, North Carolina

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The collard (*Brassica oleracea* var. *acephala*) is a kind of large kale, belonging to the headless cabbage group, Miller (1934). It is a very adaptable vegetable which may be grown on a wide diversity of soil types. It will withstand a greater temperature range, both heat and cold, than any other vegetable grown in the South. Severe frost is commonly reputed to improve its flavor and it will stand through the usual southern winter with only moderate protection from the cold. For this reason, the collard is the most important green vegetable found in southern gardens and on southern markets during the winter months.

Unlike many other vegetables, the collard has not been studied extensively; however, the available literature indicates that it surpasses or compares favorably in nutritive content with other similarly used vegetables, such as cabbage, turnip greens, and spinach. Raw ascorbic acid values previously reported from this laboratory (1944), based on the average of nine varieties, are 115 mg. per 100 grams before frost and 108 mg. per 100 grams after frost.

In the South, collards are usually cooked for a long period of time in a large quantity of water. Collards are a rich source of vitamin C, and vitamin C may be destroyed or dissolved by long cooking in large quantities of water. McKay (1943) reported a 32 per cent retention of ascorbic acid with 58 to 62 per cent solution when 170 grams of collards were cooked in 850 ml. of boiling water for 15 minutes; while Capps and Flanagan (1943) reported a 50 per cent retention when 25 grams of collards were boiled one-half hour in 200 ml. of water. This study was set up to determine the effect of two cooking methods on vitamin C in collards.

EXPERIMENTAL PROCEDURE

Source. Four varieties of collards, Herring, Morris, Vates, and Wonder, were used in the experiment. The collards were grown by the Horticulture Department. They were all harvested at the same time and kept in chests of crushed ice for use throughout the experiment.

Sampling. The edible portion of the collard is a green rosette of leaves which resembles a cabbage plant just previous to heading. On harvesting the plant for use, some growers cut the head or rosette from the stalk,

[1] Read before the Division of Agricultural and Food Chemistry, American Chemical Society meeting, Atlantic City, New Jersey, April 15, 1947.

[2] Approved for publication as Paper No. 275 of the Journal Series of the North Carolina Experiment Station.

[3] This project was conducted as a part of the National Cooperative Project, Conservation of Nutritive Value of Foods.

while other growers prefer to cut the lower leaves and allow the bud or heart leaves to continue their growth. In this study, individual heads were used. To secure representative samples for all analyses, the following sampling technique was adopted: After the damaged outer leaves were removed and discarded, the leaves were pulled off the collard head one by one and the midribs and petioles cut out with a sharp knife. One-half of each leaf was put into one pile and the other half in another pile. One of these piles was cooked, the other sliced quickly into small pieces and mixed well for the raw sample determinations of moisture and ascorbic acid.

Analyses Made. Determinations for vitamin C were made on the raw vegetable just before cooking, the vegetable immediately after cooking, and the cooking water. These analyses afforded a measure of vitamin C content of the raw vegetable, loss due to cooking, and loss of vitamin C into the cooking water. Since the collards for the entire experiment were harvested at the same time and kept in crushed ice until used, the raw values on each experimental day provided data for the calculation of any storage losses. A portion of the cooked collards was held in an aluminum pan at room temperature for twenty minutes following cooking and then analyzed for ascorbic acid. This was done in order to gain some idea of the stability of the ascorbic acid content from the end of cooking to the time when it would usually be eaten.

Cooking Methods. Two cooking methods were studied. In the pressure cooking method, three hundred grams of collards were placed in a four-quart pressure saucepan containing one-half cup (approximately 118 ml.) of water. The pressure indicator on the pan was brought to "cook" and kept on "cook" for five minutes.[4] In the boiling method, 200 grams of collards were placed in an aluminum pan with loosely fitting cover containing one quart of boiling water (enough water to cover the raw collards) and boiled one-half hour.

Sampling After Cooking. The cooked product was drained, cut up quickly with a sharp knife to insure getting a representative sample, and analyzed for ascorbic acid and moisture. The volume, weight, and ascorbic acid content of the cooking water were determined.

Methods of Analysis. The ascorbic acid was determined by the Heinze-Kanapaux (1944) indophenol method, using a Coleman Universal Spectrophotometer Model 11. No attempt was made to determine dehydroascorbic acid. The moisture content was found by drying 30-gram samples at approximately 100°C.(212°F.) for 24 hours at atmospheric pressure.

Design of Experiment. Because of the number of variables involved, all of the cookings could not be performed in one day. Differences due to varieties, pans, days, or storage in ice, and cooking methods had to be considered in order to obtain comparable results. The experiment was designed as two four by four Latin squares, one Latin square for each cooking method. This meant that each variety was sampled on each of eight experimental days, the eight experimental days falling within the

[4] The manufacturer claims that at "cook" the pressure is 15 pounds and temperature 250°F.

period of February 22 to March 12, 1946. There were four pressure saucepans and four regular type saucepans. Each variety was cooked in each of the pans, the assignment of varieties to pans being random, except for the stipulation that each variety be cooked once and only once on each experimental day, and once and only once in each experimental pan. All data were analyzed by the analysis of variance, in order to determine whether the observed differences were real or due merely to experimental variation in the method of study. In the comparisons on the cooking of vegetables, the per cent retention in the vegetable immediately after cooking, calculated from values on a fresh weight basis, was used in the statistical treatment. In the comparison of holding data, ascorbic acid on a dry weight basis was used, and in the comparison of storage losses, the fresh raw values were used.

RESULTS AND DISCUSSION

Ascobric Acid Content of Raw Collards. The initial values, or values before any storage on ice, for the varieties in mg. per 100 grams were: Herring, 157; Morris, 92; Vates, 129; and Wonder, 136. From the analysis of variance, the differences between varieties were highly significant. The least significant difference between varieties at the five per cent level was 17.5 milligrams and at the one per cent level, 23.1 milligrams. Thus, on the basis of the means, there was no real difference between Herring, Vates, and Wonder, but Morris was definitely lower. During the storage period there was a small but steady loss in ascorbic acid in Herring, Vates, and Wonder, the average loss being linear at the rate of about 1.7 milligrams per 100 grams per day. Morris, which had a comparatively low initial value, showed a slight gain (0.21 mg. per 100 grams per day) during the storage period.

Effect of Cooking. The effect of cooking on the ascorbic acid content of collards is found (Table 1). From the analyses of variance (Table 2) based on per cent retention and solution, there were no significant differ-

TABLE 1

Effect of Cooking on Ascorbic Acid of Collards

	Retention	Solution	Loss
Pressure Saucepan [1]	*pct.*	*pct.*	*pct.*
Herring	58	8	34
Morris	64	6	30
Vates	71	5	24
Wonder	71	8	21
Average	66	7	27
Covered Pan [2]			
Herring	18	58	25
Morris	22	53	26
Vates	24	50	25
Wonder	22	64	14
Average	21	56	23

[1] Three hundred grams collards cooked in one-half cup water for five minutes on "cook" presumably at 15 pounds' pressure.

[2] Two hundred grams collards cooked in one quart water for 30 minutes.

TABLE 2

Analysis of Variance

Source of variation	d.f.	Mean square			
		Presto cooker		Boiled	
		Retention	Solution	Retention	Solution
Days	3	86.90	1.21	76.06	617.25
Pans	3	257.73[1]	2.92	1.06	170.25
Varieties	3	165.56	7.51[1]	34.06	140.78
Remainder	6	46.40	1.27	19.40	303.33

[1] Significant at the five per cent level.

ences, except for the mean square for pans in per cent retention during cooking in the pressure saucepans, and the mean square for varieties in per cent solution during cooking in the pressure saucepans. These were significant at the five per cent level. The large mean square for pans indicates that there was a pan difference, i.e., that the results obtained in a pressure saucepan depend somewhat on the pan used. This is not surprising, because slight leaks and small differences in the setting of the pressure indicator, safety cap, and sealing band may cause differences in the pressure and temperature during cooking.

TABLE 3

Ascorbic Acid Content of Foods[1]

(Mg./100 gm.—Consumption Basis)

Collards, cooked in a pressure saucepan	76
Lemon Juice, fresh	60
Strawberries	50
Orange juice, fresh	50
Orange juice, canned	45
Cabbage, young, green, raw	40
Grapefruit juice, fresh	40
Grapefruit juice, canned	30
Tomato juice, fresh	30
Tomato juice, canned	25
Collards, boiled one-half hour	24
Lettuce, green leaf	10

[1] Bessey, O. A., 1939. The Vitamins, A.M.A. Symposium, 372-374.

From the data it appears that the apparent destruction of vitamin C during cooking by the two methods is about the same. On a consumption basis there is a real difference between the two cooking methods (Tables 1 and 3). In pressure cooking, the vegetable as eaten retains 66 per cent of its original ascorbic acid (approximately 76 milligrams per 100 grams); however, in boiling, the vegetable retains only 21 per cent of its original ascorbic acid (24 milligrams per 100 grams). Thus, collards cooked in a pressure saucepan are an excellent source of vitamin C, while boiled collards are a fair source of vitamin C. Use of the cooking water could compensate for this discrepancy, but is impractical when large quantities of water are used.

Effect of Holding. Although the values after holding were nearly always lower than those immediately after cooking, loss in vitamin C when collards were held at room temperature for 20 minutes was not significant (Table 4). In contrast, McKay (1943) found that holding collards at a high temperature in a double boiler was detrimental to vitamin C.

TABLE 4

Effect of Standing at Room Temperature on Ascorbic Acid in Cooked Collards

(Mg./gm., dry basis)

	Immediately after cooking	20 minutes after cooking
Pressure saucepan		
Herring	6.6	5.1
Morris	5.6	5.5
Vates	7.3	6.8
Wonder	7.3	7.1
Covered pan		
Herring	3.1	2.2
Morris	3.2	3.6
Vates	3.7	2.9
Wonder	3.6	3.6

SUMMARY

Comparisons have been made of ascorbic acid retentions in four varieties of collards when cooked in pressure saucepans and when boiled in covered pans. Thirty-two cookings were made during a period of three weeks on uniform material stored on ice. The experiment was designed to eliminate any effects due to pans, days of cooking, and storage.

Initial ascorbic acid values of the raw collards were as follows (in milligrams per 100 grams): Herring, 157; Morris, 92; Vates, 129; and Wonder, 136. Upon storage on ice for three weeks, the collards lost an average of 1.7 milligrams per 100 grams per day. When cooked in a pressure saucepan for five minutes, the average retention obtained immediately after cooking for all varieties was 66 per cent; seven per cent was dissolved in the cooking liquid and 27 per cent lost. When boiled in a covered pan for one-half hour, the average retention for all varieties was 21 per cent; 56 per cent was dissolved in the cooking liquid and 23 per cent lost.

Differences in retention, solution, and destruction between varieties were very small. When the freshly-cooked material was allowed to stand for 20 minutes, at room temperature, there was no serious loss in ascorbic acid, regardless of variety or the method of cooking.

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STORAGE LOSSES OF BUTTERNUT SQUASHES[1]

ARTHUR D. HOLMES
Massachusetts Agricultural Experiment Station,
Amherst, Massachusetts

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The normal crop of squashes in this locality is more than twice the volume that can be absorbed by the ultimate consumers before freezing weather arrives. Consequently, a large tonnage of squashes is placed annually in storage for use during the winter months. Since the Blue Hubbard, *Curcurbita maxima,* is grown most extensively, various studies regarding desirable storage conditions have been made with this variety of squash. Considerable information has been accumulated and distributed concerning the requisites for the construction and operation of squash houses, for obtaining satisfactory storage of squashes during the low-temperature winter months. Stuart (1908) studied the moisture and decay losses of Hubbard squash in storage and concluded that squashes properly grown and handled may be held until midwinter, with reasonable assurance that moisture and decay losses will be covered by the increased price received. Cummings and Jenkins (1925) recommend temperatures of 5.6 to 11.1°C.(42 to 52°F.) as optimum for storing Hubbard squash.

In studying local conditions, Boyd (1932) found that black rot of squashes was caused by the fungus, *Mycosphaerella citrullina,* but probably Kauffman (1921) was the first to identify *Mycosphaerella citrullina* as the cause. Ramsey, Wiant, and Link (1938) commented upon two diseases, *Choanephora curcurbitarum* and *Mycosphaerella citrullina,* that affect the squash fruits. Guba (1940) has noted three types of fungi which attack winter squashes during storage, i.e., *Mycosphaerella citrullina, Bacillus traheiphilus,* and *Penicillium sp.* Yeager, Richards, and Phillips (1945) reported four causes of decay of squashes during storage, but these were not identical with those reported by Guba. These authors stated that when Blue Hubbard squashes were stored at temperatures of 15.56 to 21.11°C.(60 to 70°F.) and relative humidities of 30 to 50 per cent, the organisms causing losses were black rot (*Mycosphaerella citrullina*), soft rot (*Rhizopus nigricans*), dry rot (*Fusarium sp.*), and bacterial soft rot (*Erwinia trachephila*). Black rot was the most important disease causing losses.

While these and other investigations supply a large volume of data regarding desirable storage conditions and data regarding storage losses of Hubbard squashes (*Curcurbita maxima*), a survey of the literature revealed only a little data concerning storage losses of Butternut squash (*Cucurbita moschata*). During a study of the composition of Butternut squashes at different stages of storage, data were assembled regarding moisture and decay losses. In view of the paucity of data concerning

[1] Contribution No. 653, Massachusetts Agricultural Experiment Station.

storage conditions and storage losses of Butternut squashes, and in view of the rapidly expanding production of Butternut squashes in this locality, these data are reported at this time.

EXPERIMENTAL WORK

One hundred and thirty well-colored, fully matured Butternut squashes were placed in storage, October 13, 1947, in an open laboratory attic where the temperature and relative humidity were quite constant. It was planned to start the study at harvest-time with Butternut squashes grown by Pearson [2] from seed produced by a single plant of known ancestry during the 1946 season, but an unexpected frost ended this program. Subsequently, the squashes discussed below were donated by Young [3] from a supply produced during cultural and genetic studies of Butternut squashes. When the squashes were received at the laboratory they were washed to remove extraneous soil, dried, and the tip of the stem end dipped in molten paraffin. Since, under practical conditions, squashes may be stored in full sunshine in greenhouses or in dark squash houses, the experimental squashes were divided into two comparable lots. During the nine-week experimental period the two lots, separated from each other by a one-half-inch compoboard partition, were stored, one continuously in the dark and the other continuously in the light. Light was provided by four 20-inch, white fluorescent electric bulbs suspended beneath a 46- by 49-inch metal reflector 24 inches above the squashes. Temperature and relative humidity were continuously recorded with a Friez automatic recording hygro-thermograph. The extreme temperatures were 11 and 24°C.(51.8 and 75.2°F.). Obviously, the higher temperatures occurred during October and the lower temperatures during December, but for nearly 60 per cent of the experimental period the temperature remained between 15 and 20°C.(59 and 68°F.). Since the fluorescent bulbs were suspended 24 inches above the floor on which the squashes rested, they did not increase the temperature of the air surrounding the squashes. Daily temperature readings showed only about one degree of difference in the temperature of the air surrounding the two lots of squashes and the higher temperature alternated at irregular intervals between the two lots of squashes. The extreme values for relative humidity were 26 and 52 per cent, but for over 89 per cent of the nine weeks the relative humidity ranged between 30 and 40 per cent.

The temperature conditions in this study were practically identical to those recommended by Boyd (1933) for commercial winter storage of squashes. The relative humidity was also well within Boyd's (1947) suggestion to commercial squash growers that the relative humidity of the squash storage quarters be maintained below 50 per cent. When placed in storage the squashes weighed from 609 to 2,001 gm., with an average weight of 1,192 gm.

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[2] Pearson, O. H. Eastern States Farmers' Exchange, Field Station, Feeding Hills, Massachusetts.

[3] Young, R. E. Massachusetts Agricultural Experiment Station, Waltham Field Station, Waltham, Massachusetts.

RESULTS AND DISCUSSION

Data essential for interpreting the results of the study are reported (Table 1). The approximately identical average weights for the squashes of the two lots was a natural consequence of carefully matching the initial weights of each pair of squashes as they were assigned to the lot to be stored in the dark, and to the lot to be stored in the light. The subsequent weights of the squashes during the experimental period showed a continuous though not regular rate of loss of weight throughout the storage period. Inasmuch as the two lots of squashes were stored within two feet of each other and were separated by only a one-half-inch movable, temporary partition, it is not clear why the weight losses should have been so different, i.e., 20.3 per cent for the squashes stored in the dark, and 15.8 per cent for the squashes stored in the light.

TABLE 1

Storage Losses of Butternut Squash

Length of experiment	Start	3 weeks	5 weeks	7 weeks	9 weeks	Total
	Loss in weight					
Squashes stored in the dark						
Average weight (gm.)	1190.9	1097.3	1062.4	1029.2	949.6	241.3
Per cent loss	.....	7.9	2.9	2.8	6.7	20.3
Squashes stored in the light						
Average weight (gm.)	1192.5	1110.4	1070.4	1054.1	1004.1	188.4
Per cent loss	.....	6.9	3.3	1.3	4.2	15.8
	Loss through decay					
Squashes stored in the dark						
Per cent loss	.....	10.9	7.8	26.6	24.6	69.9
Squashes stored in the light						
Per cent loss	.....	1.6	25.0	35.9	15.4	77.9

These losses in the weight of Butternut squashes are of somewhat the same order as those reported by Phillips (1946), who found a 11.15 per cent loss during the first two weeks of storage and a 25.05 per cent loss during three months' storage. In Young's (1946) studies Butternut squashes lost 12 to 14 per cent in weight between October 6 and December 19, with a total loss of 20 per cent up to January 3, and he concluded that unless a means can be found to reduce the shrinkage, Butternut squash cannot be stored economically. From a later study Young (1947) reported the loss in weight of Butternut squash as 29 per cent at the end of 14 weeks, and stated that dipping the squash in wax emulsion at harvest, or two weeks later, reduced the loss to 17.5 per cent. No other data regarding loss of weight of Butternut squashes during storage were found in the literature consulted.

Considered from the commercial grower's financial returns, these storage-weight-losses are excessive as compared with those for Blue Hubbard squashes. Phillips (1946) compared storage losses for Butternut and Blue Hubbard squashes and found the latter lost 3.19 per cent of their original weight during the first two weeks of storage; 9.72 per cent during three months'; and 16.06 per cent during the six months' storage. Yeager and

associates (1945) report similar storage-weight-losses for Blue Hubbard squashes, i.e., five per cent in 14 days, 13 per cent in 97 days, and 15 per cent in 170 days.

Decay losses during storage of the Butternut squashes included in this study were excessive. While the decay losses were very large at each of the two weeks' intervals, it will be noted (Table 1) that the largest losses for both the squashes stored in the dark and in the light were at the end of seven weeks' storage, i.e., 26.6 and 35.9 per cent respectively. Obviously, the total losses, 69.9 and 77.9 per cent, from decay, are a strong substantiation of the commercial grower's empirical observations that it is financially ruinous to store Butternut squashes for more than a short time after harvest.

Pearson,[4] Yeager,[5] and Young [6] report from their personal experiences that Butternut squash is not ordinarily a long keeper, and that under usual storage conditions the major portion have rotted by Christmas. These excessive decay losses are in sharp contrast to the relatively small decay losses of Hubbard squash during storage. For instance, Stuart (1908) reports only 4.8 per cent loss from this cause during four months' storage.

In considering the data reported (Table 1), it will be noted that the decay losses were larger for the squashes stored in the light. While continuous light may increase the rate of decay of Butternut squashes, it was not possible to determine whether or not the extent of fungus infection was equal for the two lots of squashes when they were placed in storage. Thus, it is impossible from the data assembled here to draw an accurate conclusion regarding the influence of light on the rate of decay of Butternut squashes during storage. However, the chances for infection were equally great in both lots.

Almost without exception the cause of decay of the squashes was black rot (*Mycosphaerella citrullina*). In practically all instances the decay started at the tip of the neck where the squashes had been attached to the vine, but in a very few instances it started on the lower neck or bulb of the squash at sites of injury. Boyd (1939) found the fungus, *Mycosphaerella citrullina,* infects the leaves, vines, and runners of squashes, melons, and cucumbers in the field, and that the field infection of the fruit progresses through the stalk into the neck of the squash, causing stem-end rot while the squashes are in storage. He further states that the early manifestation of black rot occurs about four to six weeks after the squashes are harvested. Since the decay started at the stem end of the squashes under discussion and progressed through the long neck towards the bulb end, it seems probable that the squash vines were infected during the growing stage; that the fungus was present in the fruit stem and the necks of the experimental squashes were already infected with *Mycosphaerella citrullina* when they were harvested. In their study of this condition, Yeager,

[4] Personal communication, Pearson, O. H. Eastern States Farmers' Exchange, Field Station, Feeding Hills, Massachusetts.

[5] Personal communication, Yeager, A. F. Department of Horticulture, New Hampshire Agricultural Experiment Station, Durham, New Hampshire.

[6] Personal communication, Young, R. E. Massachusetts Agricultural Experiment Station, Waltham Field Station, Waltham, Massachusetts.

Richards, and Phillips (1945) cut the stems of the Hubbard squashes at varying lengths and subsequently recommended that the stem be completely removed, since that will minimize black rot and Fusarium dry rot. Guba (1946) dipped Butternut squash in an emulsion that consisted of two quarts of formaldehyde, 20 gallons of water, and three gallons of Ceremul wax, and reduced shrinkage considerably, but did not reduce the amount of decay; and Yeager, Richards, and Phillips (1945) applied liquid and dust fungicides to squashes at harvest-time in an attempt to prevent entrance of fungus into squash tissue but these did not prove valuable for controlling storage rot, thus supporting Boyd's (1939) contention that infection enters by the stalk. However, Guba (1947) made a recent study of the effect of field applications of fungicides on disease control and yield of Butternut squashes. He compared Fermate, Phygon, Zerlate, Bordeaux, tribasic copper sulfate, and no treatment. The percentage of uninfected fruits at harvest in the sprayed plots varied from 3.5 to 9.1 per cent, and in the unsprayed plots from 18.5 to 22.7 per cent. The best control was obtained with Phygon, but the total yield and average weight of squashes were least in the Phygon-sprayed plots.

SUMMARY

Two comparable lots of well-developed, fully mature Butternut squashes grown under experimental conditions were placed in storage on October 13, 1947. Since squashes are commercially stored in green houses or in dark squash houses, one lot was stored in the dark and the second in continuous, uniform, artificial light. The two lots were about two feet apart and separated by only a one-half-inch temporary partition. The temperature during the nine-week experimental period was 15 to 20°C.(59 to 68°F.) and the relative humidity ranged between 30 and 40 per cent. The weight losses of the squashes stored in the dark were 7.9, 2.9, 2.8, and 6.7 per cent, respectively, at the 3-, 5-, 7-, and 9-week intervals, and the total weight loss was 20.3 per cent. The corresponding losses for the squashes stored in the light were 6.9, 3.3, 1.3, and 4.2 per cent, with a total loss of 15.8 per cent. The decay losses, at the different intervals for the squashes stored in the dark, were 10.9, 7.8, 26.6, and 24.6 per cent, or a total of 69.9 per cent. The corresponding losses for the squashes stored in the light were 1.6, 25.0, 35.9, and 15.4 per cent, and totaled 77.9 per cent. The decay was largely, if not wholly, due to black rot (*Mycosphaerella citrullina*).

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SIGNIFICANCE IN TRIANGULAR TASTE TESTS

E. B. ROESSLER, J. WARREN, AND J. F. GUYMON
University of California, Davis, California

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In testing some samples for flavor some differences are obvious. In other samples differences are less easily determined. The "triangular test"[1] or odd sample method, is useful in comparing two samples which are almost alike. In this test the taster is served, at the same time, three samples identified only by letters or numbers and is aware that two of the samples are identical, the other different. He is asked to answer two questions: "Can you detect a difference?" and "Which two are alike?" He may also be asked which he prefers and whether he can describe the difference.

There are six possible orders in which the samples may be presented: AAB, ABA, BAA, BBA, BAB, ABB. If the difference is open to doubt, the tests should be repeated until each taster has been served the samples in each of these possible sequences. Although this method is open to the usual criticisms of any subjective test, it does lend itself to statistical analysis. It can be used first with an expert panel to determine whether a difference exists and then with a large number of people to determine consumer acceptability of either or both samples. It also may be employed for selecting personnel for expert panels, wherein individual sensitivities to different taste factors are evaluated.

Since the test is triangular, the probability p of a taster guessing the right sequence is one-third, and the probability q of an incorrect guess is two-thirds. By chance alone the expected numbers of correct answers, therefore, for n tasters would be n/3 and the number of incorrect 2n/3. The standard error of the distribution $= \sqrt{npq} = \sqrt{2n/3}$. If y denotes the observed number of correct answers, then the normal deviate, applying Yate's Correction for Continuity, would be

$$\frac{y - \frac{n}{3} - 0.5}{\sqrt{\frac{2n}{3}}}$$

At the five, one, and one-tenth per cent levels of significance the values of this ratio are, respectively, 1.960, 2.576, and 3.291. This leads to three proportions for determining the values of y at these three levels of significance:

[1] Helm, Erick, and Trolle, Birger, 1946. Selection of a taste panel. Wallerstein Laboratories Communications 9, 181-194.

TABLE 1

Probability in Triangular Taste Tests

No. of tasters or tastings	No. of correct answers necessary to establish significant differentiation			No. of tasters or tastings	No. of correct answers necessary to establish significant differentiation		
	P=0.05	P=0.01	P=0.001		P=0.05	P=0.01	P=0.001
7	5	6	7	57	27	29	31
8	6	7	8	58	27	29	32
9	6	7	8	59	27	30	32
10	7	8	9	60	28	30	33
11	7	8	9	61	28	30	33
12	8	9	10	62	28	31	33
13	8	9	10	63	29	31	34
14	9	10	11	64	29	32	34
15	9	10	12	65	30	32	35
16	10	11	12	66	30	32	35
17	10	11	13	67	30	33	36
18	10	12	13	68	31	33	36
19	11	12	14	69	31	34	36
20	11	13	14	70	32	34	37
21	12	13	15	71	32	34	37
22	12	14	15	72	32	35	38
23	13	14	16	73	33	35	38
24	13	14	16	74	33	36	39
25	13	15	17	75	34	36	39
26	14	15	17	76	34	36	39
27	14	16	18	77	34	37	40
28	15	16	18	78	35	37	40
29	15	17	19	79	35	38	41
30	16	17	19	80	35	38	41
31	16	18	19	81	36	38	41
32	16	18	20	82	36	39	42
33	17	19	20	83	37	39	42
34	17	19	21	84	37	40	43
35	18	19	21	85	37	40	43
36	18	20	22	86	38	40	44
37	18	20	22	87	38	41	44
38	19	21	23	88	39	41	44
39	19	21	23	89	39	42	45
40	20	22	24	90	39	42	45
41	20	22	24	91	40	42	46
42	21	22	25	92	40	43	46
43	21	23	25	93	40	43	46
44	21	23	25	94	41	44	47
45	22	24	26	95	41	44	47
46	22	24	26	96	42	44	48
47	23	25	27	97	42	45	48
48	23	25	27	98	42	45	49
49	23	25	28	99	43	46	49
50	24	26	28	100	43	46	49
51	24	26	29	200	80	84	89
52	25	27	29	300	117	122	127
53	25	27	29	400	152	158	165
54	25	27	30	500	188	194	202
55	26	28	30	1,000	363	372	383
56	26	28	31	2,000	709	722	737

At the five per cent level,

$$\frac{y - \frac{n}{3} - 0.5}{\sqrt{\frac{2n}{3}}} = 1.960$$

$$y = \frac{n}{3} + 0.5 + 0.9239\sqrt{n}$$

At the one per cent level,

$$\frac{y - \frac{n}{3} - 0.5}{\sqrt{\frac{2n}{3}}} = 2.576$$

$$y = \frac{n}{3} + 0.5 + 1.214\sqrt{n}$$

At the one-tenth per cent level,

$$\frac{y - \frac{n}{3} - 0.5}{\sqrt{\frac{2n}{3}}} = 3.291$$

$$y = \frac{n}{3} + 0.5 + 1.551\sqrt{n}$$

The same expressions for determining y can also be derived from the X^2 distribution for a two by two contingency table.

In Table 1 are tabulated for various numbers of tests the number of correct answers, as calculated from the above expressions, at the five, one, and one-tenth per cent levels necessary to establish significant differentiation.

EFFECT OF HEAT UPON THE NUTRITIVE VALUES OF PEANUTS. I. PROTEIN QUALITY

LEDA W. BUSS[1] AND VERZ R. GODDARD
Department of Home Economics, University of California, Los Angeles, California

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High protein foods of animal origin, because of their essentially high cost of production, tend to be scarce. Since several of the legumes, including soybeans and peanuts, contain proteins approaching the nutritional value of meat proteins, they are deserving of a more important place in the diet. These foods, like meat, are nearly always cooked when used for human consumption.

This is of special interest, since it has been noted many times that processing of foods by heat may have marked effect upon the quality of the protein. Proteins of various foods, such as cereals, Morgan (1925); legumes, Hayward (1936a) and Johnson (1939); meat, Morgan (1934); and milk, Greaves and Morgan (1933) have been shown to be modified by heat treatment. Soybeans have been found to present an interesting case; the protein quality was improved by moderate cooking, Johnson, Parsons, and Steenbock (1939), but was impaired by more severe heat treatment, Parsons (1943). Very little information appears to be available at present concerning the effect of heat upon peanut proteins.

Special emphasis might well be laid on the nutritive values of peanuts, particularly since the development of a highly satisfactory peanut flour, which may be utilized in various ways. The proteins of peanuts are of particular significance, since they are present in a fairly large proportion, about 25.8 per cent in peanuts according to Holmes (1918) and 60 per cent in peanut flour according to Payne (1942). The quality of the protein has been found to be such as to make it a valuable plant source of this important food factor.

Since peanuts are almost never used in the raw state in human diets, it was decided to investigate the possible effect upon their protein value of the processing which they undergo. Three types of processing were selected for study: (1) Boiling in water for 40 minutes, which is a common method of preparing peanuts in certain foreign countries, and which also approximates the type and intensity of heat treatment involved in the preparation of certain vegetarian dishes; (2) roasting at a temperature of 160°C.(320°F.) for 40 minutes, which is common procedure in preparation of peanut butter according to von Loesecke (1942), and which also yields roasted peanuts much like those used as confections; and (3) roasting at 180°C.(356°F.) for 40 minutes, which yields a somewhat overroasted product. Tests were also planned on raw peanuts, which served as

[1] Data presented in this paper are based upon the thesis prepared by Leda W. Buss in partial fulfillment of the requirements for the Master of Science Degree in Home Economics, University of California, Los Angeles, February, 1947.

the basis for comparison with the processed products. The experimental work tested the effect of processing in these three ways by comparing the growth-promoting quality of the peanuts so treated with that of raw peanuts.

EXPERIMENTAL WORK

Peanuts tested were of the Virginia type and were purchased raw in the shell. Processing was performed on the shelled peanuts with the red skin left on. Before heat-processing, these averaged 25 per cent protein and four per cent moisture.

Boiling 500 grams of peanuts for 40 minutes in one kilogram of water caused softening of the nuts and loss of raw flavor. They were spread out on trays and dried in an oven at 50°C.(122°F.) to their original weight before grinding for use in the experimental diet.

The two roasting procedures (at 160 and 180°C.) were carried out on kilogram lots of the nuts which had first been shelled and then spread out one layer thick on pans. These were placed in an oven which had been preheated to the temperature in question. The roasting period was 40 minutes after the oven had again returned to 160 or 180°C. The peanuts roasted at the former temperature were light tan in color and had a flavor comparable to the better of the commercially roasted products; those roasted at 180°C. were dark brown and slightly unpleasant in flavor, due to overroasting. This temperature was used to exaggerate the possible differences in nutritive values of edible peanuts due to differences in heat treatment. Because of the low moisture content of peanuts, these methods of roasting caused a weight loss of less than one per cent for the 160° roasting and of less than three and one-half per cent for the 180° roasting. It was unnecessary, therefore, to correct for moisture loss in the roasted products.

Growth experiments were carried out on four groups of albino rats, 80 animals in all, so selected that (insofar as possible) litter-mates of the same sex were placed on each of four diets. Diets were planned so that peanuts were the sole source of protein and the feeding technique made possible accurate records of food intake to compare with the weight increments of the animals during the eight-week experimental period.

The four experimental diets were identical except in the preparation of the peanuts. One contained ground raw peanuts; the other three, peanuts boiled and roasted at 160 and 180°C., respectively. The composition was as follows per 100 grams: ground peanuts, 40.0 grams; salt mixture [Hubbell, Mendel, and Wakeman (1937)], 2.5 grams; sardine oil,[2] 1.2 grams; corn oil, 8.0 grams; vitamin B complex mixture,[3] 0.1 gram; and cornstarch, 48.2 grams.

The rats were fed and weighed three times a week. The false bottom cages were suspended above a shelf entirely covered with heavy butcher

[2] "Sea Pep," Fortified Sardine Oil, Van Camp Laboratories, Terminal Island, California.

[3] The vitamin mixture used successfully by Unna and others (1941). Quantities were more than sufficient for the rat if as much as five grams of diet were consumed per day.

paper and, in addition, smaller pieces slightly larger than the area of the cage floor were placed under each cage. Food was placed in small jars fitted with lids in which there was a round, smooth-edged hole sufficiently large to allow passage of the animal's head. The food cups were placed in larger jars to catch most of the food scattered by the animal. In order to keep an accurate record of food intake, food cups and their contents, together with the scatterings scraped from the scatter cup and the scatter paper below the cage, were weighed to the nearest 0.1 gram. The cup was then refilled with diet and reweighed. The difference between the weight of the full cup on one day and the weight of cup, contents, and scatterings on the succeeding weighing day, was taken as the food intake for the intervening period. Since the protein content of the diet was ten per cent, one-tenth of total food consumption could be calculated as weight of protein consumed. The grams gain in weight per gram of protein consumed was calculated for each animal and group averages found.

Lysine supplementation (one gram per day of a 2.5 per cent lysine solution) was given forcibly to a small test sample of rats on the over-roasted peanut diet, in order to study the effect of such supplementation on the growth of the animals.

TABLE 1

Growth Response of Litter-Mates to Four Types of Peanut Diets. Weight Increments per Gram Protein Consumed

Litter number	Sex	Raw	Boiled	Roasted 160°	Roasted 180°
		grams	*grams*	*grams*	*grams*
200	Male	2.12	2.29	1.85	.....
200	Female	2.17	2.37	2.13	.....
220	Male	2.14	2.23	2.11	.....
220	Female	1.99	2.18	2.00	.....
230	Male	1.72	2.00	1.23	.....
230	Male	1.90	1.84	1.44	.....
240	Male	.....	1.84	1.32	0.58
240	Female	.....	1.49	1.53	.....
250	Male	2.13	2.19	1.26	0.46
250	Female	0.74	1.87	1.20	0.49
260	Female	1.33	1.01	1.38	0.46
270	Male	.....	1.65	1.93	0.47
270	Female	1.74	2.11	1.75	0.41
280	Male	.....	.....	1.63	0.41
280	Female	.....	.....	1.56	0.63
500	Female	1.58	.....	1.38	0.39
500	Female	1.86	.....	1.96	.....
520	Male	2.03	2.04	.....	0.74[1]
520	Female	1.88	1.42	1.87	—0.25
530	Male	1.43	1.02	1.84	—0.18
530	Female	1.50	1.69	1.80	0.21[1]
540	Male	1.46	1.74	.....	.....
540	Female	1.40	.....	1.37	—0.23
550	Male	1.90	1.58	1.74	0.44[1]
560	Female	1.97	1.58	1.56	0.41[1]
570	Female	.....	1.71	.....	—0.22

[1] Weight increments per gram of protein consumed over total eight-week period, including six weeks on lysine-supplemented diet and two weeks on unsupplemented diet.

RESULTS AND CONCLUSIONS

The manner in which the experimental animals were selected for the various diets permitted comparison on the basis of individual performance of litter-mates of like sex on each diet, and also on the basis of group averages. Litter-mate comparisons have been given (Table 1), and the group averages have been recorded (Table 2).

TABLE 2

Weight Increments per Gram of Protein Consumed by Rats on Five Types of Peanut Diets

Number	Sex	Raw	Boiled	Roasted 160°	Roasted 180°	Roasted 180° lysine
		grams	*grams*	*grams*	*grams*	*grams*
1	Male	2.12	2.29	1.85	0.58	1.11[1]
2	Male	2.14	2.23	2.11	0.47	0.92[1]
3	Male	1.72	2.00	1.23	0.46	.....
4	Male	1.90	1.84	1.44	0.41	.....
5	Male	2.13	2.19	1.32	—0.18	.....
6	Male	2.03	1.65	1.26	.....	.....
7	Male	1.46	2.04	1.93	.....	.....
8	Male	1.93	1.74	1.63	.....	.....
9	Male	.....	1.58	1.84	.....	.....
10	Male	.....	1.84	1.74	.....	.....
Sum	Males	15.43	19.40	16.35	1.74	2.03
Mean	Males	1.93	1.94	1.64	0.33	1.02
1	Female	2.17	2.37	2.13	0.63	1.07[1]
2	Female	1.99	2.18	2.00	0.49	0.92[1]
3	Female	0.74	1.49	1.53	0.46	0.65[1]
4	Female	1.33	1.87	1.20	0.40	.....
5	Female	1.74	1.01	1.38	—0.22	.....
6	Female	1.58	2.11	1.75	—0.23	.....
7	Female	1.86	1.42	1.56	—0.25	.....
8	Female	1.88	1.69	1.96	—0.33	.....
9	Female	1.50	1.58	1.38	.....	.....
10	Female	1.40	1.71	1.87	.....	.....
11	Female	1.93	.....	1.80	.....	.....
12	Female	1.27	.....	1.37	.....	.....
13	Female	.....	.....	1.56	.....	.....
Sum	Females	19.39	17.43	21.49	0.95	2.64
Mean	Females	1.62	1.74	1.65	0.12	0.88
Sum	Total	34.82	36.83	37.84	2.69	4.67
Mean	Total	1.74	1.84	1.65	0.21	0.93

[1] Based on six-week experimental period.

From these tables it may be seen that the animals receiving the diets in which the sole source of protein was raw, boiled, or moderately roasted peanuts, made consistently good gains, while those on the diet containing peanuts roasted at 180° made poor gains, or losses. The mean gains of all animals per gram of protein consumed was 1.74 grams; of those on the raw peanut diet, 1.74 grams; on the boiled peanut diet, 1.84 grams; and

on the peanuts roasted at 160°, 1.65 grams. On the diet containing peanuts roasted at 180°, the mean gain was but 0.21 gram, one-eighth or less of the mean on the other three diets.

Animals given the lysine supplement made consistently superior gains compared to those on the unsupplemented highly roasted peanuts, but inferior to the gains made by animals on the other diets. In this connection it was interesting to note the gains of individual animals before and after starting the lysine supplement (Table 3). In each case the animal failed to maintain its initial weight until lysine was added to the diet. After adding lysine all animals made substantial gains.

TABLE 3

Growth Responses of Rats on Highly Roasted Peanut Diet to Lysine Supplement. Weight Increments per Gram Protein Consumed

Rat number	Sex	Unsupplemented 14 days	Supplemented 48 days	Total for 56 days
		grams	*grams*	*grams*
524	Male	—0.68	1.11	0.74
556	Male	—0.62	0.92	0.44
509	Female	—1.61	1.07	0.39
567	Female	—0.75	0.92	0.41
537	Female	—0.62	0.65	0.21

The consistently small gains, or losses, of the animals receiving the highly roasted peanut diet were so marked as to require no further analysis to establish the significance of the data. Differences among the other diets, however, were analyzed statistically. The "t" test described by Snedecor (1940) was used to make litter-mate comparisons, while group averages were treated according to the method of analysis of variance for unweighted class numbers, Snedecor (1940). Although the weight increments on the boiled peanut diet appeared to be slightly superior to those on the other diets, these statistical analyses revealed that the differences were not significant.

These results would indicate that while there was an apparent slight improvement in protein quality when raw peanuts were boiled for forty minutes such differences were not (as in the case of soybeans) statistically significant. Nor did roasting at 160°C.(320°F.) for forty minutes cause any true alteration in protein quality. More severe heat treatment [roasting at 180°C.(356°F.)], however, occasioned a change which impaired the usefulness of the protein in promoting growth in young rats. The immediate and marked growth responses of the animals given a lysine supplement to the highly roasted peanut diet, suggested that this amino acid might be at least one of the limiting factors of the processed proteins.

SUMMARY

The effect of heat upon quality of peanut proteins was studied by means of rat growth, using 80 albino rats. The diets contained 10 per cent protein (40 per cent peanuts) and differed from one another only with regard to heat treatment of the peanuts which were (a) roasted at 160°C., (b) roasted at 180°C., (c) boiled, and (d) untreated (raw).

Results indicated that the growth promoting values of the raw peanuts, boiled peanuts, and those roasted at 160°C. were not significantly different. On these diets the average weight increments per gram of protein consumed were 1.74, 1.84, and 1.65 grams, respectively. However, the rats on the peanuts roasted at 180°C. showed an average gain of only 0.21 gram per gram of protein consumed, which differed so greatly from the growth of animals on the raw and moderately roasted peanuts that statistical treatment was not required for proof of its significance. Lysine supplementation to the diets containing the overroasted peanuts brought about a significant improvement in growth.

It may be concluded from these results that moderate heat treatment, either by moist heat or roasting, does not alter significantly the nutritive quality of peanut proteins. Overroasting has deleterious effects, and peanuts roasted to a dark brown color may be so impaired that their proteins are essentially worthless for growth production.

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EFFECT OF HEAT UPON THE NUTRITIVE VALUES OF PEANUTS. II. RIBOFLAVIN AND PANTOTHENIC ACID CONTENT

KATHARINE REMINGTON DUNN[1] AND VERZ R. GODDARD
Department of Home Economics, University of California, Los Angeles, California

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The problem of maintaining the people of devastated areas of the world, as well as the population of the United States, on a well-balanced, adequate diet has appeared even more difficult of solution recently than during the latter months of the war. As has been pointed out in the first paper of this series, certain of the legumes, especially soybeans and peanuts, deserve attention because of the relatively high quality of their proteins. The extensive research on the B-vitamins during the past few years has shown that the legumes are valuable for these factors, as well as for protein.

There has been considerable variation, however, in the reported riboflavin content of peanuts, while information concerning the pantothenic acid content of peanuts and other legumes is very limited in quantity. Moreover, data on vitamins in overroasted peanuts, and in the nuts cooked by methods other than roasting, have not been collected.

Supplee, Bender, and Jensen (1939) found riboflavin values ranging from 450 to 470 μg. per 100 gm. of raw peanuts, while Cheldelin and Williams (1943) reported 110, and Daniel and Norris (1945) obtained an average of 165 μg. Sherman (1946) tabulated the range of riboflavin values in roasted peanuts as 100 to 350 μg. per 100 gm. The only published values on the pantothenic acid content are those of Cheldelin and Williams (1943) who reported 2,500 μg. per 100 gm. of raw peanuts.

The investigation reported in this paper deals with the riboflavin and pantothenic acid content of raw peanuts and the effect of processing methods upon these vitamins.

EXPERIMENTAL WORK

Heat treatments of the peanuts were essentially identical to the processes reported in Paper I, Protein Quality. Whole, Virginia-type peanuts were treated in three ways. In order to obtain a product corresponding in color and flavor to commercially roasted peanuts, von Loesecke (1942), 500 gm. of peanuts with skins remaining, were roasted one layer deep in a previously heated oven at 160°C.(320°F.) for 40 minutes. A second batch was roasted at 180°C.(356°F.) for the same period so that an overheated product was obtained such as those sold after reheating for sale. Because of the practice, in certain parts of the world, of boiling peanuts, in the third procedure 500 gm. of peanuts were plunged into a liter of boiling

[1] Data presented in this paper are based upon the thesis prepared by Katharine Remington Dunn in partial fulfillment of the requirements for the Master of Science degree in Home Economics, University of California at Los Angeles, June, 1946.

water for forty minutes. They were then dried for 18 hours at 50°C. (122°F.) to restore them to their original moisture content. Raw peanuts were found to contain only four per cent moisture as discussed in the first paper on Protein Quality, and since roasting them by the two methods resulted in weight loss of less than one per cent for the peanuts roasted at 160°C., and of less than 3.5 per cent for those roasted at 180°C., it was decided that this moisture content and loss was well within the experimental error of the assay method. Hence, no correction was made in calculating the final results. All the peanuts were ground and stored in brown glass bottles for future use.

The microbiological method of Snell and Strong (1939) employing *Lactobacillus casei* ε was used in the assay of riboflavin, and that of Pennington, Snell, and Williams (1940), using the same organism, for pantothenic acid.

Because of the stimulatory effect of fat upon the growth of *L. casei*, it was removed from the ground peanuts by extracting in a Soxhlet extractor for six hours with diethyl ether. Enzymatically digested samples were used for the assays of both riboflavin and pantothenic acid. Two methods of riboflavin extraction were used. In Method I an aliquot of defatted peanuts was digested by a buffered solution of takadiastase and papain, as recommended by Cheldelin, Eppright, Snell, and Guirard (1942). By Method II, the peanuts were autoclaved in a large amount of water in accordance with the method of Snell and Strong (1939) and then digested by takadiastase as used by Cheldelin *et al.* (1942). This second method was used in the preparation of peanuts for the pantothenic acid assay, also.

In order to determine the effect of processing, triplicate sample tubes containing basal medium plus extracts prepared from raw peanuts, boiled peanuts, those roasted at 160°C., and at 180°C., were inoculated with the organism and incubated for a three-day period at 37°C.(98.6°F.). In the case of both the riboflavin and the pantothenic acid assays, the lactic acid produced by *L. casei* was titrated electrometrically to a pH of 6.8 to 7.0 by means of a Beckman pH meter. Results were plotted on standard curves obtained by similar titrations of lactic acid in inoculated tubes of known riboflavin and pantothenic acid content.

Recovery experiments with added riboflavin and pantothenic acid were included in two assays. Riboflavin recoveries ranged from 98 to 105 per cent, indicating that the vitamin was not lost during preparation, and that any stimulatory or inhibitory effect of the assay materials was too small to be significant. Recoveries of pantothenic acid ranged from 89 to 97 per cent. It is evident that some pantothenic acid was lost in the assay process, but the amount was too small to be significant.

Range of values obtained for riboflavin and for pantothenic acid in peanuts before and after processing are shown (Table 1); percentage losses are given also (Table 2).

RESULTS AND DISCUSSION

When comparing the mean riboflavin and pantothenic acid content of raw peanuts obtained (Table 1) with those of other workers, it is necessary to consider several important factors: the type of peanut assayed, the

preparation of the peanuts, i.e., whether the skins were allowed to remain, and the method of extracting the vitamins from the ground peanuts.

Riboflavin values for raw peanuts obtained by Cheldelin and Williams of 110 μg. per 100 gm. agree best with the figures presented (Table 1), in which 98 and 96 μg. of riboflavin per 100 gm., respectively, were found for the two extraction methods. Type of peanut employed by these investigators was not stated. Pantothenic acid values for raw peanuts reported by these workers (2,500 μg. per 100 gm.) were also in good agreement with those shown (Table 1)—2,715 μg. per 100 gm. The riboflavin value of 165 ug. per 100 gm. reported by Daniel and Norris was obtained from assays of white Spanish and Runner peanuts.

TABLE 1

Riboflavin and Pantothenic Acid Values of Raw and Heated Peanuts[1]

Treatment of peanuts	Method of preparing assay materials	Number of weighed samples	Range of values	Mean value with P.E.
			μg. per 100 gm.	*μg. per 100 gm.*
I. Riboflavin				
Raw (none)	Method I	8	152-61	98± 7.91
Boiled	Method I	8	103-56	81± 3.86
Roasted 160°C.	Method I	7	113-68	97± 4.60
Roasted 180°C.	Method I	6	85-57	68± 2.86
Raw (none)	Method II	12	110-80	96± 2.13
Boiled	Method II	9	108-45	72± 5.29
Roasted 160°C.	Method II	10	124-80	98± 3.01
Roasted 180°C.	Method II	9	100-60	79± 3.57
II. Pantothenic acid				
Raw (none)	Method II	7	3120-2120	2715±83.9
Boiled	Method II	7	2320-1800	2051±43.9
Roasted 160°C.	Method II	6	2240-1920	2069±32.8
Roasted 180°C.	Method II	7	280-160	225±12.9

[1] Values used in these tables were retained after applying "Casting-out" principle used by Swift (1939) to outstanding values.

The riboflavin value of 450 to 470 μg. per 100 gm. of raw peanuts reported by Supplee *et al.* is at variance with those given above, but may possibly be accounted for by the extraction method used, or by some other variation in technique.

Column 4 (Table 1) indicates there is some difference in the mean riboflavin values obtained for raw and processed peanuts attributable to the extraction technique employed. This difference is slight, however, and the two sets of figures were averaged in calculating the mean values and percentage losses presented (Table 2).

Findings from assays of both vitamins are summarized (Table 2). The mean riboflavin value of raw peanuts was found to be 97 ± 4.92 μg. per 100 gm.; that for pantothenic acid was 2,715 ± 83.9 μg. per 100 gm. Roasting the peanuts at 160°C. for 40 minutes in the amounts specified, as recommended by von Loesecke, produced a product similar in color and flavor to those produced commercially. It was found, however, that manufacturers of peanut butter chose quite variable roasting conditions. Miller, Louis, and Peterson (1943) reported that temperatures used in the com-

mercial roasting of peanuts ranged from 121°C.(249.8°F.) for 50 to 60 minutes to 343.3°C.(650°F.) for 45 to 60 minutes. Such a wide range could be accounted for only by a consideration of such factors as the size of batch of peanuts roasted, size of the oven relative to the load it contained, manner in which the peanuts were distributed, and amount of moisture in the peanuts. In our own experimental work, too, the length of time it was necessary to roast raw peanuts to obtain a uniform product varied when 1,000 and 500 gms. were used.

TABLE 2

Percentage Losses of Riboflavin and Pantothenic Acid Due to Heating [1]

Method of processing	Riboflavin [2]		Pantothenic acid	
	Mean value with P.E.	Loss	Mean value with P.E.	Loss
	μg. per 100 gm.	*pct.*	*μg. per 100 gm.*	*pct.*
Raw (none)	97±4.92	....	2715±83.9	....
Boiled	77±4.57	21	2051±43.9	24
Roasted 160°C.	98±3.80	0	2069±32.8	24
Roasted 180°C.	74±3.21	24	225±12.9	92

[1] The significance of the differences between the raw and processed peanuts was determined by using the "t-test" as recommended by Snedecor (1940); "t" is calculated by using the following formula:

$$t = \frac{x - m}{S\bar{x}}$$: where x = mean difference;

m = o; and $S\bar{x}$ = the standard error of the differences.

[2] Average riboflavin values obtained with Methods I and II are used in this table.

The mean riboflavin value for peanuts roasted at 160°C. was 98 ± 3.80 μg. per 100 gm. No loss due to the roasting process was found. On the contrary, there seemed to be a tendency for the riboflavin values of these peanuts to exceed those obtained for raw peanuts. Our explanation of the slightly higher values for peanuts roasted at 160°C. is that the heating process may have increased the ease of extraction of the vitamin or its availability to the organism. Lantz (1939) advanced this theory to explain why cooked pinto beans assayed by the rat growth method were found to be higher in riboflavin than the raw beans.

In evaluating the three methods of processing peanuts, results indicate that roasting the peanuts at a moderate temperature, such as 160°C., retains the riboflavin and pantothenic acid content to a greater extent than the other two methods. When the peanuts were boiled, approximately one-fourth of both vitamins was lost. Roasting them at a higher temperature, i.e. 180°C., incurred a loss of one-fourth the riboflavin content and approximately nine-tenths of the pantothenic acid content of the peanuts.

In drawing conclusions, it is necessary to know whether peanuts, following favorable processing, would be a valuable addition to the diet in respect to these two vitamins. In comparing the riboflavin content of peanuts roasted at 160°C. with the riboflavin values of foods commonly used in the American diet, they were found to contain one-half the amount present in milk,[2] an almost equal amount to that of whole-grain

[2] Tabulated by Bowes and Church (1946).

wheat and bread products,[3] but were considerably lower than riboflavin values reported for fresh meat, which ranged from 260 μg per 100 gm. of beef muscle to 320 for lamb muscle.[4]

Comparison of pantothenic acid values of peanuts roasted at 160°C. with figures obtained for other foods is limited by scarcity of published data. Cooperman and Elvehjem (1944), using the microbiological method, obtained values ranging from 1,290 to 1,690 μg. per 100 gm. in the assay of groats and rolled oats, which are considerably lower than those reported for raw peanuts. Peanuts were also found to compare very favorably with fresh meat figures reported by Waisman and Elvehjem (1941).

From the above data it is evident that peanuts are a fair source of riboflavin and a very good, or excellent, source of pantothenic acid following favorable processing. Considering their fuel value and content of high quality proteins, along with the factors reported, it would seem appropriate from a nutritional standpoint to advocate to a much greater extent than is now being done the use of peanuts in the American dietary and in the depleted diets of citizens of foreign countries.

SUMMARY

Microbiological assays for riboflavin and pantothenic acid were performed on Virginia-type raw peanuts. Other samples of the same peanuts were assayed following boiling, roasting at 160°C., and roasting at 180°C. for a 40-minute period.

The mean riboflavin value of raw peanuts was found to be 97 ± 4.92 μg. per 100 gm. and that for pantothenic acid, 2,715 ± 83.9 μg. per 100 gm.

Boiling the peanuts produced a loss of approximately one-fourth of both riboflavin and pantothenic acid; while roasting them at 180°C. destroyed one-fourth the riboflavin and almost all the pantothenic acid.

Roasting them at 160°C. was the most favorable processing method, since none of the riboflavin and only one-fourth of the pantothenic acid was lost.

It was concluded that peanuts are a fair source of riboflavin and a very good, or excellent, source of pantothenic acid following favorable processing.

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[3] Tabulated by Sherman (1946).

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